#12

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Robert J. D'Amato

Serial No.: **09/545,139**

Filed: **April 7, 2000**

For: **Methods and Compositions For The Inhibition of Angiogenesis With EM-138**

Art Unit: **1614**

Examiner: **Goldberg, J.**

FAX RECEIVED
FEB 2 5 2002
PETITIONS OFFICE

INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

Sir:

The citation of information on the attached Form PTO/SB/08A, "List of Art Cited by Applicant" is made pursuant to 37 C.F.R. §§ 1.97 and 1.98. A copy of each cited item is enclosed unless stated otherwise herein below.

The serial numbers of the below-listed U.S. patent applications are listed on this form as provided in M.P.E.P. §609(D), rather than a PTO/SB/08A form, to avoid the serial numbers of the pending U.S. patent applications being published on any patent that may issue from this application.

Applicant does not waive the confidential nature of any of the below-listed patent applications.

I hereby certify that this correspondence is being sent by facsimile transmission to the Assistant Commissioner For Patents, to facsimile number (703) 308-6916, on February 25, 2002

Jeffery B. Arnold - Reg. No. 39,540

ATLLIB02 72814.1

_____ U.S. Patent Application Serial No. 09/578,845, filed May 25, 2000.

_____ U.S. Patent Application Serial No. 09/547,087, filed April 11, 2000.

_____ U.S. Patent Application Serial No. 09/704,054, filed November 1, 2000.

_____ U.S. Patent Application Serial No. 09/710,533, filed November 9, 2000.

_____ U.S. Patent Application Serial No. 09/899,318, filed July 5, 2001.

_____ U.S. Patent Application Serial No. 09/966,895, filed September 28, 2001.

_____ U.S. Patent Application Serial No. 09/899,344, filed July 5, 2001.

_____ U.S. Patent Application Serial No. 10/020,391, filed December 12, 2001.

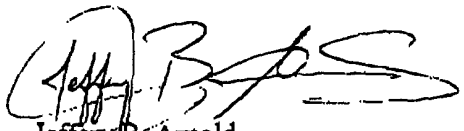
_____ U.S. Patent Application Serial No. 10/015,252, filed December 12, 2001.

_____ U.S. Patent Application Serial No. 10/026,291, filed December 20, 2001.

The citation of this information does not constitute an admission that any of the materials are available as a reference or of priority, or a waiver of any right applicant may have under applicable statutes, Rules of Practice in patent cases, or otherwise.

No additional fees are believed due, however, the Commissioner is hereby authorized to charge any deficiencies which may be required or credit any overpayment to Deposit Account Number 11-0855.

Respectfully submitted,



Jeffery B. Arnold
Reg. No. 39,540

KILPATRICK STOCKTON LLP
1100 Peachtree Street
Suite 2800
Atlanta, Georgia 30309-4530
(404) 815-6500
Our Docket: 05213-0650 (43170-219681)

US006316471B1

(12) **United States Patent**
Muller et al.

(10) **Patent No.: US 6,316,471 B1**
(45) **Date of Patent: Nov. 13, 2001**

(54) **ISOINDOLINES, METHOD OF USE, AND PHARMACEUTICAL COMPOSITIONS**

(75) Inventors: **George W. Muller**, Bridgewater; **David I. Stirling**, Branchburg; **Roger Shen-Chu Chen**, Edison, all of NJ (US)

(73) Assignee: **Celgene Corporation**, Warren, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 17 days.

(21) Appl. No.: **09/634,061**

(22) Filed: **Oct. 17, 2000**

Related U.S. Application Data

(62) Continuation of application No. 09/543,809, filed on Apr. 6, 2000, now Pat. No. 6,281,230, which is a division of application No. 09/230,389, filed as application No. PCT/US97/13375 on Jul. 24, 1997, now abandoned, which is a continuation of application No. 08/701,494, filed on Aug. 22, 1996, now Pat. No. 5,798,368, which is a continuation of application No. 08/690,258, filed on Jul. 24, 1996, now Pat. No. 5,635,517.

(60) Provisional application No. 60/048,278, filed on May 30, 1997.

(51) Int. Cl.[7] A61K 31/454; C07D 401/04

(52) U.S. Cl. 514/323; 546/201

(58) Field of Search 514/323; 546/201

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,590,189	5/1986	Hiraga et al. .
4,808,402	2/1989	Leibovich et al. .
4,849,441	7/1989	Okazaki et al. .
5,385,901	1/1995	Kaplan et al. .
5,463,063	10/1995	Muller .
5,502,066	3/1996	Heinemann et al. .
5,593,990	1/1997	D'Amato .
5,605,914	2/1997	Muller .
5,629,327	5/1997	D'Amato .
5,635,517	6/1997	Muller et al. .
B 5,635,517	6/1999	Muller et al. .
5,658,940	8/1997	Muller et al. .
5,698,579	12/1997	Muller .
5,703,098	12/1997	Muller et al. .
5,712,291	1/1998	D'Amato .
5,728,845	3/1998	Muller et al. .
5,736,570	4/1998	Muller et al. .
5,798,368	8/1998	Muller et al. .
5,801,195	9/1998	Muller et al. .
5,874,448	2/1999	Muller et al. .
5,877,200	3/1999	Muller .
5,929,117	7/1999	Muller et al. .
5,968,945	10/1999	Muller et al. .
6,011,050	1/2000	Muller et al. .
6,020,358	2/2000	Muller et al. .
6,046,221	4/2000	Muller et al. .
6,071,948	6/2000	D'Amato .

FOREIGN PATENT DOCUMENTS

WO 95/01348	1/1995	(WO) .

OTHER PUBLICATIONS

Corral, L. et al., "Differential Cytokine Modulation and T Cell Activation by Two Distinct Classes of Thalidomide Analogues That Are Potent Inhibitors of TNF-1", The Journal of Immunology, pp. 380-386, 1999.

Corral, L. et al., "Immunomodulation by thalidomide and thalidomide analogues", Annals of the Rheumatic Diseases, vol. 58, Suppl. 1, pp. 1107-1113, 1999.

Corral, L. et al., "Selection of Novel Analogs of Thalidomide with Enhanced Tumor Necrosis Factor Inhibitory Activity", Molecular Medicine, vol. 2, No. 4, 1996.

Craig, J.C., "Absolute Configuration of the Enantiomers of 7-Chloro-4-[[4-diethylamino)-1-methylbutyl]amino] quinoline (chloroquine)", J. Org. Chem., vol. 53, pp. 1167-1170, 1988.

Feiser, Louis F., Experiments in Organic Chemistry, 3rd edition, p. 75, 1955.

He, Weixuan et al, "Synthesis of Thalidomide Analogs and Their Biological Potential for Treatment of Graft Versus Host Disease (GVHD)", 206th American Chemical Society National Meeting, Med. Chem. Abst. 216, 1993.

Koch, H., The Arene Oxide Hypothesis of Thalidomide Action. Considerations on the Molecular Mechanism of Action of the "Classical" Teratogen", Sci. Pharm., vol. 49, pp. 67-99 (1981).

Koch, H., 4 Thalidomide and Congeners as Anti-Inflammatory Agents, Progress in Medicinal Chemistry, vol. 22, pp. 166-242 (1985).

Miyachi, H. et al., Novel Biological Response Modifiers: Phthalimides with Tumor Necrosis Factor-ProductionRegulating Activity, J. Med. Chem., pp. 2858-2865 (1997).

Miyachi, H. et al., Tumor Necrosis Factor-Alpha Production Enhancing Activity of Substituted 3'-Methylthalidomide: Influence of Substituents at the Phthaloyl Moiety on the Activity of Stereoselectivity, Chem. Pharm. Bull., 46(7), pp. 1165-1168 (1998).

Muller, George et al., Amino-Substituted Thalidomide Analogs: Potent Inhibitors of TNF-Production, Bioorganic & Medicinal Chem. Letters 9, pp. 1625-1630 (1999).

(List continued on next page.)

Primary Examiner—Charanjit S. Aulakh
(74) *Attorney, Agent, or Firm*—Mathews, Collins, Shepherd & Gould, P.A.

(57) **ABSTRACT**

Substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and 1-oxo-2-(2,6-dioxopiperidin-3-yl)isoindolines reduce the levels of TNFα in a mammal. Typical embodiments are 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline and 1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-aminoisoindoline.

26 Claims, No Drawings

OTHER PUBLICATIONS

Niwayama, Satomi et al., Potent Inhibition of Tumor Necrosis Factor–Production by Tetrafluorothalidomide and Tetrafluorophthalimides, J. Med. Chem., pp. 3044–3045 (1996).

Smith, R. L. et al., Studies on the Relationship Between the Chemical Structure and Embryotoxic Activity of Thalidomide and Related Compounds, Symp. Embryopathic Act. Drugs, pp. 194–209 (1965).

Jonsson, N., Chemical Structure and Teratogenic Properties, Acta. Pharm. Suicica, vol. 9 pp. 521–542 (1972).

Muller, George et al., Structural Modifications of Thalidomide Produce Analogs with Enhanced Tumor Necrosis Factor Inhibitory Activity, Journal of Medicinal Chemistry, vol. 39, No. 17, pp. 3238–3240(1996).

Muller, George et al., Thalidomide Analogs and PDE4 Inhibition, Bioorganic & Medicinal Chemisty Letters 8, pp. 2669–2674 (1998).

Niwayama, Satomi et al., Enhanced Potency of Perfluorinated Thalidomide Derivatives for Inhibition of LPS–Induced Tumor Necrosis Factor–Production is Associated with a Change of Mechanism of Action, Bioorganic & Medicinal Chemistry Letters 7, pp. 1071–1076 (1998).

Shannon, Edward J. et al., Immunomodulatory Assays to Study Structure–Activity Relationships of Thalidomide, Immunopharmacology 35, pp. 203–212 (1997).

Takeuchi, Yoshio et al., (R)–and (S)–Fluorothalidomides: Isosteric Analogues of Thalidomide, American Chemical Society, vol. 1, No. 10, pp. 1571–1573 (1999).

Udagawa, Taturo et al., Thalidomide and Analogs, Antiangiogenia Agents in Cancer Therapy, pp. 263–274.

Zwingenberger, K. et al., Immunomodulation by Thalidomide: Systematic Review of the Literature and of Unpublished Observations, Journal of Inflammation, pp. 177–211 (1996).

ISOINDOLINES, METHOD OF USE, AND PHARMACEUTICAL COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of Ser. No. 09/543,809 filed Apr. 6, 2000 now U.S. Pat. No. 6,281,230, which is a divisional of Ser. No. 09/230,389, now abandoned, which is based on PCT/US97/13375 filed Jul. 24, 1997, which is a continuation of Ser. No. 08/690,258 filed Jul. 24, 1996, now U.S. Pat. No. 5,635,517, Ser. No. 08/701,494 filed Aug. 22, 1996, now U.S. Pat. No. 5,798,368, and provisional application Ser. No. 60/048,278 filed May 30, 1997.

DETAILED DESCRIPTION

The present invention relates to substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolines, the method of reducing levels of tumor necrosis factor α in a mammal through the administration thereof, and pharmaceutical compositions of such derivatives.

BACKGROUND OF THE INVENTION

Tumor necrosis factor α, or TNFα, is a cytokine which is released primarily by mononuclear phagocytes in response to a number immunostimulators. When administered to animals or humans, it causes inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Excessive or unregulated TNFα production thus has been implicated in a number of disease conditions. These include endotoxemia and/or toxic shock syndrome {Tracey et al, *Nature* 330, 662–664 (1987) and Hinshaw et al., *Circ. Shock* 30, 279–292 (1990)}; cachexia {Dezube et al., *Lancet*, 335 (8690), 662 (1990)} and Adult Respiratory Distress Syndrome where TNFα concentration in excess of 12,000 pg/mL have been detected in pulmonary aspirates from ARDS patients {Millar et al., *Lancet* 2(8665), 712–714 (1989)}. Systemic infusion of recombinant TNFα also resulted in changes typically seen in ARDS {Ferrai-Baliviera et al., *Arch. Surg.* 124(12), 1400–1405 (1989)}.

TNFα appears to be involved in bone resorption diseases, including arthritis. When activated, leukocytes will produce bone-resorption, an activity to which the data suggest TNFα contributes. {Bertolini et al., *Nature* 319, 516–518 (1986) and Johnson et al., *Endocrinology* 124(3), 1424–1427 (1989).} TNFα also has been shown to stimulate bone resorption and inhibit bone formation in vitro and in vivo through stimulation of osteoclast formation and activation combined with inhibition of osteoblast function. Although TNFα may be involved in many bone resorption diseases, including arthritis, the most compelling link with disease is the association between production of TNFα by tumor or host tissues and malignancy associated hypercalcemia {*Calci. Tissue Int.* (*US*) 46(Suppl.), S3–10 (1990)}. In Graft versus Host Reaction, increased serum TNFα levels have been associated with major complication following acute allogenic bone marrow transplants {Holler et al., *Blood*, 75(4), 1011–1016 (1990)}.

Cerebral malaria is a lethal hyperacute neurological syndrome associated with high blood levels of TNFα and the most severe complication occurring in malaria patients. Levels of serum TNFα corelated directly with the severity of disease and the prognosis in patients with acute malaria attacks {Grau et al., *N. Engl. J. Med.* 320(24), 1586–1591 (1989)}.

Macrophage-induced angiogenesis TNFα is known to be mediated by TNFα. Leibovich et al. {*Nature*, 329, 630–632 (1987)} showed TNFα induces in vivo capillary blood vessel formation in the rat cornea and the developing chick chorioallantoic membranes at very low doses and suggest TNFα is a candidate for inducing angiogenesis in inflammation, wound repair, and tumor growth. TNFα production also has been associated with cancerous conditions, particularly induced tumors {Ching et al., *Brit. J. Cancer*, (1955) 72, 339–343, and Koch, *Progress in Medicinal Chemistry*, 22, 166–242 (1985)}.

TNFα also plays a role in the area of chronic pulmonary inflammatory diseases. The deposition of silica particles leads to silicosis, a disease of progressive respiratory failure caused by a fibrotic reaction. Antibody to TNFα completely blocked the silica-induced lung fibrosis in mice {Pignet et al., *Nature*, 344:245–247 (1990)}. High levels of TNFα production (in the serum and in isolated macrophages) have been demonstrated in animal models of silica and asbestos induced fibrosis {Bissonnette et al., *Inflammation* 13(3), 329–339 (1989)}. Alveolar macrophages from pulmonary sarcoidosis patients have also been found to spontaneously release massive quantities of TNFα as compared with macrophages from normal donors {Baughman et al., *J. Lab. Clin. Med.* 115(1), 36–42 (1990)}.

TNFα is also implicated in the inflammatory response which follows reperfusion, called reperfusion injury, and is a major cause of tissue damage after loss of blood flow {Vedder et al., *PNAS* 87, 2643–2646 (1990)}. TNFα also alters the properties of endothelial cells and has various pro-coagulant activities, such as producing an increase in tissue factor pro-coagulant activity and suppression of the anticoagulant protein C pathway as well as down-regulating the expression of thrombomodulin {Sherry et al., *J. Cell Biol.* 107, 1269–1277 (1988)}. TNFα has pro-inflammatory activities which together with its early production (during the initial stage of an inflammatory event) make it a likely mediator of tissue injury in several important disorders including but not limited to, myocardial infarction, stroke and circulatory shock. Of specific importance may be TNFα-induced expression of adhesion molecules, such as intercellular adhesion molecule (ICAM) or endothelial leukocyte adhesion molecule (ELAM) on endothelial cells {Munro et al., *Am. J. Path.* 135(1), 121–132 (1989)}.

TNFα blockage with monoclonal anti-TNFα antibodies has been shown to be beneficial in rheumatoid arthritis {Elliot et al., *Int. J. Pharmac.* 1995 17(2), 141–145} and Crohn's disease (von Dullemen et al., *Gastroenterology*, 1995 109(1), 129–135}.

Moreover, it now is known that TNFα is a potent activator of retrovirus replication including activation of HIV-1. {Duh et al., *Proc. Nat. Acad. Sci.* 86, 5974–5978 (1989); Poll et al., *Proc. Nat. Acad. Sci.* 87, 782–785 (1990); Monto et al., *Blood* 79, 2670 (1990); Clouse et al., *J. Immunol.* 142, 431–438 (1989); Poll et al., *AIDS Res. Hum. Retrovirus*, 191–197 (1992)}. AIDS results from the infection of T lymphocytes with Human Immunodeficiency Virus (HIV). At least three types or strains of HIV have been identified, i.e., HIV-1, HIV-2 and HIV-3. As a consequence of HIV infection, T-cell mediated immunity is impaired and infected individuals manifest severe opportunistic infections and/or unusual neoplasms. HIV entry into the T lymphocyte requires T lymphocyte activation. Other viruses, such as HIV-1, HIV-2 infect T lymphocytes after T cell activation and such virus protein expression and/or replication is mediated or maintained by such T cell activation. Once an activated T lymphocyte is infected with HIV, the T lympho-

cyte must continue to be maintained in an activated state to permit HIV gene expression and/or HIV replication. Cytokines, specifically TNFα, are implicated in activated T-cell mediated HIV protein expression and/or virus replication by playing a role in maintaining T lymphocyte activation. Therefore, interference with cytokine activity such as by prevention or inhibition of cytokine production, notably TNFα, in an HIV-infected individual assists in limiting the maintenance of T lymphocyte caused by HIV infection.

Monocytes, macrophages, and related cells, such as kupffer and glial cells, also have been implicated in maintenance of the HIV infection. These cells, like T cells, are targets for viral replication and the level of viral replication is dependent upon the activation state of the cells. {Rosenberg et al., *The Inmunopathogenesis of HIV Infection*, Advances in Immunology, 57 (1989)}. Cytokines, such as TNFα, have been shown to activate HIV replication in monocytes and/or macrophages {Poli et al., *Proc. Natl. Acad. Sci.*, 87, 782–784 (1990)}, therefore, prevention or inhibition of cytokine production or activity aids in limiting HIV progression for T cells. Additional studies have identified TNFα as a common factor in the activation of HIV in vitro and has provided a clear mechanism of action via a nuclear regulatory protein found in the cytoplasm of cells (Osborn, et al., *PNAS* 86 2336–2340). This evidence suggests that a reduction of TNFα synthesis may have an antiviral effect in HIV infections, by reducing the transcription and thus virus production.

AIDS viral replication of latent HIV in T cell and macrophage lines can be induced by TNFα {Folks et al., *PNAS* 86, 2365–2368 (1989)}. A molecular mechanism for the virus inducing activity is suggested by TNFα's ability to activate a gene regulatory protein (NFκB) found in the cytoplasm of cells, which promotes HIV replication through binding to a viral regulatory gene sequence (LTR) {Osborn et al., *PNAS* 86, 2336–2340 (1989)}. TNFα in AIDS associated cachexia is suggested by elevated serum TNFα and high levels of spontaneous TNFα production in peripheral blood monocytes from patients {Wright et al., *J. Immunol.* 141(1). 99–104 (1988)}. TNFα has been implicated in various roles with other viral infections, such as the cytomegalia virus (CMV), influenza virus, adenovirus, and the herpes family of viruses for similar reasons as those noted.

The nuclear factor κB (NFκB) is a pleiotropic transcriptional activator (Lenardo, et al., *Cell* 1989, 58, 227–29). NFκB has been implicated as a transcriptional activator in a variety of disease and inflammatory states and is thought to regulate cytokine levels including but not limited to TNFα and also to be an activator of HIV transcription (Dbaibo, et al., *J. Biol. Chem.* 1993, 17762–66; Duh et al., *Proc. Natl. Acad. Sci.* 1989, 86,. 5974–78; Bachelerie et al., *Nature* 1991, 350, 709–12; Boswas et al., *J. Acquired Immune Deficiency Syndrome* 1993, 6, 778–786; Suzuki et al., *Biochem. And Biophys. Res. Comm.* 1993, 193, 277–83; Suzuki et al., *Biochem. And Biophys. Res Comm.* 1992, 189, 1709–15; Suzuki et al., *Biochem. Mol. Bio. Int.* 1993, 31(4), 693–700; Shakhov et al., *Proc. Natl. Acad. Sci. USA* 1990, 171, 35–47; and Staal et al., *Proc. Natl. Acad. Sci. USA* 1990, 87, 9943–47). Thus, inhibition of NFκB binding can regulate transcription of cytokine gene(s) and through this modulation and other mechanisms be useful in the inhibition of a multitude of disease states. The compounds described herein can inhibit the action of NFκB in the nucleus and thus are useful in the treatment of a variety of diseases including but not limited to rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, septic shock, septis, endotoxic shock, graft versus host disease, wasting, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythrematosis, ENL in leprosy, HIV, AIDS, and opportunistic infections in AIDS. TNFα and NFκB levels are influenced by a reciprocal feedback loop. As noted above, the compounds of the present invention affect the levels of both TNFα and NFκB.

Many cellular functions are mediated by levels of adenosine 3',5'-cyclic monophosphate (cAMP). Such cellular functions can contribute to inflammatory conditions and diseases including asthma, inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future*, 17(9), 799–807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNFα and NFκB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle.

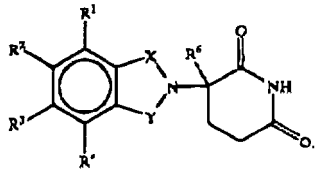
Decreasing TNFα levels and/or increasing cAMP levels thus constitutes a valuable therapeutic strategy for the treatment of many inflammatory, infectious, immunological, and malignant diseases. These include but are not restricted to septic shock, sepsis, endotoxic shock, hemodynamic shock and sepsis syndrome, post ischemic reperfusion injury, malaria, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, oncogenic or cancerous conditions, asthma, autoimmune disease, opportunistic infections in AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythrematosis, ENL in leprosy, radiation damage, oncogenic conditions, and hyperoxic alveolar injury. Prior efforts directed to the suppression of the effects of TNFα have ranged from the utilization of steroids such as dexaamethasone and prednisolone to the use of both polyclonal and monoclonal antibodies {Beutler et al., *Science* 234, 470–474 (1985); WO 92/11383}.

DETAILED DESCRIPTION

The present invention is based on the discovery that certain classes of nonpolypeptide compounds more fully described herein decrease the levels of TNFα.

In particular, the invention pertains to (i) compounds of the formula:

I



in which:

one of X and Y is C=O and the other of X and Y is C=O or CH_2;

(i) each of R^1, R^2, R^3, and R^4, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms or (ii) one of R^1, R^2, R^3, and R^4 is —NHR^5 and the remaining of R^1, R^2, R^3, and R^4 are hydrogen;

R^5 is hydrogen or alkyl of 1 to 8 carbon atoms;

R^6 is hydrogen, alkyl of 1 to 8 carbon atoms, benzyl, or halo;

provided that R^6 is other than hydrogen if X and Y are C=O and (i) each of R^1, R^2, R^3, and R^4 is fluoro or (ii) one of R^1, R^2, R^3, or R^4 is amino; and

(b) the acid addition salts of said compounds which contain a nitrogen atom capable of being protonated.

A preferred group of compounds are those of Formula I in which each of R^1, R^2, R^3, and R^4, independently of the others, is halo, alkyl of 1 to 4 carbon atoms, or alkoxy of 1 to 4 carbon atoms, and R^6 is hydrogen, methyl, ethyl, or propyl. A second preferred group of compounds are those of Formula I in which one of R^1, R^2, R^3, and R^4 is $-NH_2$, the remaining of R^1, R^2, R^3, and R^4 are hydrogen, and R^6 is hydrogen, methyl, ethyl, or propyl.

Unless otherwise defined, the term alkyl denotes a univalent saturated branched or straight hydrocarbon chain containing from 1 to 8 carbon atoms. Representative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, and tert-butyl. Alkoxy refers to an alkyl group bound to the remainder of the molecule through an ethereal oxygen atom. Representative of such alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy, and tert-butoxy. Preferably R^1, R^2, R^3, and R^4 are chloro, fluoro, methyl or methoxy.

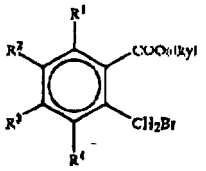
The compounds of Formula I are used, under the supervision of qualified professionals, to inhibit the undesirable effects of TNFα. The compounds can be administered orally, rectally, or parenterally, alone or in combination with other therapeutic agents including antibiotics, steroids, etc., to a mammal in need of treatment.

The compounds of the present invention also can be used topically in the treatment or prophylaxis of topical disease states mediated or exacerbated by excessive TNFα production, respectively, such as viral infections, such as those caused by the herpes viruses, or viral conjunctivitis, psoriasis, atopic dermatitis, etc.

The compounds also can be used in the veterinary treatment of mammals other than humans in need of prevention or inhibition of TNFα production. TNFα mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted above, but in particular viral infections. Examples include feline immunodeficiency virus, equine infectious anaemia virus, caprine arthritis virus, visna virus, and maedi virus, as well as other lentiviruses.

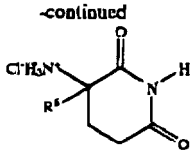
Compounds in which one of R^1, R^2, R^3, R^4 is amino and R^5 and R^6, as well as the remainder of R^1, R^2, R^3, R^4, are hydrogen, as for example, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)4-aminoisoindoline or 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline are known. See, e.g., Jönsson, *Acta Pharma. Succica*, 9, 521–542 (1972).

The compounds can be prepared using methods which are known in general. In particular, the compounds can be prepared through the reaction of 2,6-dioxopiperidin-3-ammonium chloride, and a lower alkyl ester of 2-bromomethylbenzoic acid in the presence of an acid acceptor such as dimethylaminopyridine or triethylamine.



+

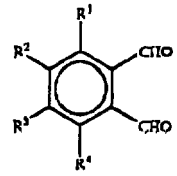
-continued



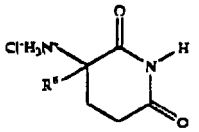
I

The substituted benzoate intermediates are known or can be obtained though conventional processes. For example, a lower alkyl ester of an ortho-toluic acid is brominated with N-bromosuccinimide under the influence of light to yield the lower alkyl 2-bromomethylbenzoate.

Alternatively, a dialdehyde is allowed to react with 2,6-dioxopiperidin-3-ammonium chloride:

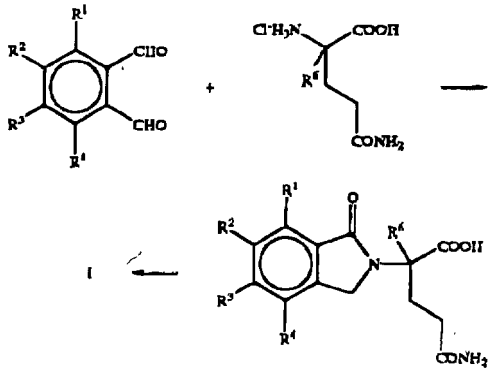


+

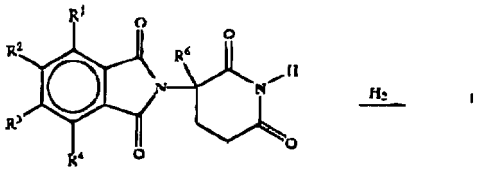


I

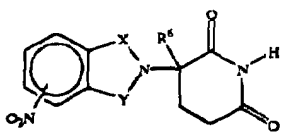
In a further method, a dialdehyde is allowed to react with glutamine and the resulting 2-(1-oxoisoindolin-2-yl)glutaric acid then cyclized to yield a 1-oxo-2-(2,6-dioxopiperidin-3-yl)-isoindoline of Formula I:



Finally, an appropriately substituted phthalidimide intermediate is selectively reduced:



Amino compounds can be prepared through catalytic hydrogenation of the corresponding nitro compound:



The nitro intermediates of Formula IA are known or can be obtained though conventional processes. For example, a nitrophthalic anhydride is allowed to react with α-aminoglutarimide hydrochloride (alternatively named as 2,6-dioxopiperidin-3-ylammonium chloride) in the presence of sodium acetate and glacial acetic acid to yield an intermediate of Formula IA in which X and Y are both C=O.

In a second route, a lower alkyl ester of nitro-ortho-toluic acid is brominated with N-bromosuccinimide under the influence of light to yield a lower alkyl 2-(bromomethyl) nitrobenzoate. This is allowed to react with 2,6-dioxopiperidin-3-ammonium chloride in, for example, dimethylformamide in the presence of triethylamine to yield an intermediate of Formula II in which one of X is C=O and the other is CH_2.

Alternatively, if one of R_1, R_2, R_3, and R_4 is protected amino, the protecting group can be cleaved to yield the corresponding compound in which one of R_1, R_2, R_3, and R_4 is amino. Protecting groups utilized herein denote groups which generally are not found in the final therapeutic compounds but which are intentionally introduced at some stage of the synthesis in order to protect groups which otherwise might be altered in the course of chemical manipulations. Such protecting groups are removed at a later stage of the synthesis and compounds bearing such protecting groups thus are of importance primarily as chemical intermediates (although some derivatives also exhibit biological activity). Accordingly the precise structure of the protecting group is not critical. Numerous reactions for the formation and removal of such protecting groups are described in a number of standard works including, for example, "Protective Groups in Organic Chemistry", Plenum Press, London and New York, 1973; Greene, Th. W. "Protective Groups in Organic Synthesis". Wiley, New York, 1981; "The Peptides", Vol. I, Schröder and Lubke, Academic Press, London and New York, 1965; "Methoden der organischen Chemie". Houben-Weyl, 4th Edition. Vol.15/I, Georg Thieme Verlag, Stuttgart 1974, the disclosures of which are incorporated herein by reference. An amino group can be protected as an amide utilizing an acyl group which is selectively removable under mild conditions, especially benzyloxycarbonyl, formyl, or a lower alkanoyl group which is branched in 1- or α position to the carbonyl group, particularly tertiary alkanoyl such as pivaloyl, a lower alkanoyl group which is substituted in the position α to the carbonyl group, as for example trifluoroacetyl.

The compounds of the present invention possess a center of chirality and can exist as optical isomers. Both the racemates of these isomers and the individual isomers themselves, as well as diastereomers when there are two chiral centers, are within the scope of the present invention. The racemates can be used as such or can be separated into their individual isomers mechanically as by chromatography using a chiral adsorbent. Alternatively, the individual isomers can be prepared in chiral form or separated chemically from a mixture by forming salts with a chiral acid, such as the individual enantiomers of 10-camphorsulfonic acid, camphoric acid, α-bromocamphoric acid, methoxyacetic acid, tartaric acid, diacetyltartaric acid, malic acid, pyrrolidone-5-carboxylic acid, and the like, and then freeing one or both of the resolved bases, optionally repeating the process, so as obtain either or both substantially free of the other; i.e., in a form having an optical purity of >95%.

The present invention also pertains to the physiologically acceptable non-toxic acid addition salts of the compounds of Formula I. Such salts include those derived from organic and inorganic acids such as, without limitation, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid, citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embonic acid, enanthic acid, and the like.

The compositions preferably are formulated in unit dosage for, meaning physically discrete units suitable as a unitary dosage, or a predetermined fraction of a unitary dose to be administered in a single or multiple dosage regimen to human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with a suitable pharmaceutical excipient The compositions can be formulated so as to provide an immediate, sustained or delayed release of active ingredient after administration to the patient by employing procedures well known in the art.

Oral dosage forms include tablets, capsules, dragees, and similar shaped, compressed pharmaceutical forms containing from 1 to 100 mg of drug per unit dosage. Isotonic saline solutions containing from 20 to 100 mg/mL can be used for parenteral administration which includes intramuscular, intrathecal, intravenous and intra-arterial routes of administration. Rectal administration can be effected through the use of suppositories formulated from conventional carriers such as cocoa butter.

Pharmaceutical compositions thus comprise one or more compounds of the present invention associated with at least one pharmaceutically acceptable carrier, diluent or excipient. In preparing such compositions, the active ingredients are usually mixed with or diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule or sachet. When the excipient serves as a diluent, it may be a solid, semi-solid, or liquid material which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders. Examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidinone, cellulose. water, syrup, and methyl cellulose, the formulations can additionally include lubricating agents such as talc, magnesium stearate and mineral oil, wetting agents, emulsifying and suspending agents, preserving agents such as methyl- and propylhydroxybenzoates, sweetening agents or flavoring agents.

The following examples will serve to further typify the nature of this invention but should not be construed as a limitation in the scope thereof, which scope is defined solely by the appended claims.

EXAMPLE 1

1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline

A mixture of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline (alternatively named as N-(2,6-

dioxopiperidin-3-yl)-4-nitrophthalimide} (1 g, 3.3 mmol) and 10% Pd/C (0.13 g) in 1,4-dioxane (200 mL) was hydrogenated at 50 psi for 6.5 hours. The catalyst was filtered through Celite and the filtrate concentrated in vacuo. The residue was crystallized from ethyl acetate (20 mL) to give 0.62 g (69%) of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline {alternatively named as N-(2,6-dioxopiperidin -3-yl)-4-aminophthalimide} as an orange solid. Recrystallization from dioxane/ethyl acetate gave 0.32 g of yellow solid: mp 318.5–320.5° C.; HPLC (nova Pak C18.15/85 acetonitrile/0.1%H_3PO_4) 3.97 min (98.22%). ^1H NMR (DMSO-d_6) δ 11.08(s, 1H), 7.53–7.50 (d, J=8.3 Hz, 1H), 6.94(s, 1H), 6.84–6.81(d, J=8.3 Hz, 1H), 6.55(s,2H), 5.05–4.98(m, 1H), 2.87–1.99(m 4H); ^{13}C NMR (DMSO-d_6) δ 172.79,170.16, 167.65, 167.14, 155.13, 134.21, 125.22, 116.92, 116.17, 107.05, 48.58, 30.97, 22.22; Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 56.52-H, 4.17; N, 14.60.

In a similar fashion from 1-oxo-2-(2,6dioxopiperidin-3-yl)-5-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-nitroisoindoline, and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline, there is respectively obtained 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline, and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, respectively, upon hydrogenation.

EXAMPLE 2

1,3-Dioxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline

A mixture of 4-nitrophthalic anhydride (1.7 g, 8.5 mmol), α-aminoglutarimide hydrochloride (1.4 g, 8.5 mmol) and sodium acetate (0.7 g, 8.6 mmol) in glacial acetic acid (30 mL) was heated under reflux for 17 hours. The mixture was concentrated in vacuo and the residue was stirred with methylene chloride (40 mL) and water (30 mL). The aqueous layer was separated, extracted with methylene chloride (2x40 mL). The combined methylene chloride solutions were dried over magnesium sulfate and concentrated in vacuo to give 1.4 g (54%) of 1,3dioxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline as a light brown solid. An analytical sample was obtained by recrystallization from methanol: mp 228.5–229.5° C.; ^1H NMR (DMSO-d_6) δ 11.18(s, 1H), 8.69–8.65(d,d J=1.9 and 8.0 Hz, 1H), 8.56(d, J=1.9 Hz, 1H), 8.21(d, H=8.2 Hz, 1H), 5.28(dd, J=5.3 and 12.8 Hz, 1H), 2.93–2.07(m, 4H); ^{13}C NMR (DMSO-d_6) δ 172.66, 169.47, 165.50, 165.23, 151.69, 135.70, 132.50, 130.05, 124.97, 118.34, 49.46, 30.85, 21.79; Anal. Calcd for $C_{13}H_9N_3O_6$: C, 51.49; H, 2.99; N, 13.86. Found: C, 51.59; H, 3.07; N, 13.73.

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-5-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-nitroisoindoline, and 1-oxo-2-(2,6-dioxopiperidin-3-yl)-7-nitroisoindoline can be obtained by allowing 2,6-dioxopiperidin-3-ammonium chloride to react with methyl 2-bromomethyl-5-nitrobenzoate, methyl 2-bromomethyl-4-nitrobenzoate, methyl 2-bromomethyl-6-nitrobenzoate, and methyl 2-bromomethyl-7-nitrobenzoate, respectively, in dimethylformamide in the presence of triethylamine. The methyl 2-(bromomethyl)nitrobenzoates in turn are obtained from the corresponding methyl esters of nitro-orthotoluic acids by

conventional bromination with N-bromosuccinimide under the influence of light.

EXAMPLE 3

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline

A mixture of 16.25 g of 2,6-dioxopiperidin-3-ammonium chloride, and 30.1 g of methyl 2-bromomethyl-3,4,5,6-tetrafluorobenzoate, and 12.5 g of triethylamine in 100 mL of dimethylformamide is stirred at room temperature for 15 hours. The mixture is then concentrated in vacuo and the residue mixed with methylene chloride and water. The aqueous layer is separated and back-extracted with methylene chloride. The combined methylene chloride solutions are dried over magnesium sulfate and concentrated in vacuo to give 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline.

In a similar fashion 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrachloroisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethylisoindoline, and 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisoindoline are obtained by substituting equivalent amounts of 2-bromomethyl-3,4,5,6-tetrachlorobenzoate, 2-bromomethyl-3,4,5,6-tetramethylbenzoate, and 2-bromomethyl-3,4,5,6-tetramethoxybenzoate, respectively, for 2-bromomethyl-3,4,5,6-tetrafluorobenzoate.

EXAMPLE 4

N-Benzyloxycarbonyl-α-methyl-glutamic Acid

To a stirred solution of α-methyl-D,L-glutamic acid (10 g, 62 mmol) in 2 N sodium hydroxide (62 mL) at 0–5° C. was added benzyl chloroformate (12.7 g, 74.4 mmol) over 30 min. After the addition was complete the reaction mixture was stirred at room temperature for 3 hours. During this time the pH was maintained at 11 by addition of 2N sodium hydroxide (33 mL). The reaction mixture was then extracted with ether (60 mL). The aqueous layer was cooled in an ice bath and then acidified with 4N hydrochloric acid (34 mL) to pH=1. The resulting mixture was extracted with ethyl acetate (3x100 mL). The combined ethyl acetate extracts were washed with brine (60 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to give 15.2 g (83%) of N-benzyloxycarbonyl-α-methylglutamic acid as an oil: ^1H NMR ($CDCl_3$) δ 8.73(m, 5H), 5.77(b, 1H), 5.09(s, 2H), 2.45–2.27(m, 4H), 2.0(s, 3H).

In a similar fashion from α-ethyl-D,L-glutamic acid and α-propyl-D,L-glutamic acid, there is obtained N-benzyloxycarbonyl-α-ethylglutamic acid and N-benzyloxycarbonyl -α-propylglutamic acid, respectively.

EXAMPLE 5

N-Benzyloxycarbonyl-α-methyl-glutamic Anhydride

A stirred mixture of N-benzyloxycarbonyl-α-methyl-glutamic acid (15 g, 51 mmol) and acetic anhydride (65 mL) was heated at reflux under nitrogen for 30 min. The reaction mixture was cooled to room temperature and then concentrated in vacuo to afford N-benzylcarbonyl-α-methylglutamic anhydride as an oil (15.7 g) which can be used in next reaction without further purification: ^1H NMR ($CDCl_3$) δ 7.44–7.26 (m, 5H), 5.32–5.30 (m, 2H), 5.11 (s, 1H), 2.69–2.61 (m, 2H), 2.40–2.30 (m, 2H), 1.68 (s, 3H).

In a similar fashion from N-benzyloxycarbonyl-α-ethylglutamic acid and N-benzyloxycarbonyl-α-

propylglutamic acid, there is obtained N-benzylcarbonyl-α-ethylglutamic anhydride and N-benzylcarbonyl-α-propylglutamic anhydride, respectively.

EXAMPLE 6

N-Benzyloxycarbonyl-α-methylisoglutamine

A stirred solution of N-benzylcarbonyl-α-methylglutamic anhydride (14.2 g, 1.5 mmol) in methylene chloride (100 mL) was cooled in an ice bath. Gaseous ammonia was bubbled into the cooled solution for 2 hours. The reaction mixture was stirred at room temperature for 17 hours and then extracted with water (2x50 mL). The combined aqueous extracts were cooled in an ice bath and acidified with 4N hydrochloric acid (32 mL) to pH 1. The resulting mixture was extracted with ethyl acetate (3x80 mL). The combined ethyl acetate extracts were washed with brine (60 mL) and then dried ($MgSO_4$). The solvent was removed in vacuo to give 11.5 g of N-benzyloxycarbonyl-α-amino-α-methylisoglutamine: ^1H NMR ($CDCl_3$/DMSO) δ 7.35 (m, 5H), 7.01 (s, 1H), 6.87 (s, 1H), 6.29 (s, 1H), 5.04 (s, 2H), 2.24–1.88 (m, 4H), 1.53 (s, 3H).

In a similar fashion from N-benzylcarbonyl-α-ethylglutamic anhydride and N-benzylcarbonyl-α-propylglutamic anhydride there is obtained N-benzyloxycarbonyl-α-amino-α-ethylisoglutamine and N-benzyloxycarbonyl-α-amino-α-propylisoglutamine, respectively.

EXAMPLE 7

N-Benzyloxycarbonyl-α-amino-α-methylglutarimide

A stirred mixture of N-benzyloxycarbonyl-α-methylisoglutamine (4.60 g, 15.6 mmol), 1,1'-carbonyldiimidazole (2.80 g, 17.1 mmol), and 4-dimethylaminopyridine (0.05 g) in tetrahydrofuran (50 mL) was heated to reflux under nitrogen for 17 hours. The reaction mixture was then concentrated in vacuo to an oil. The oil was slurried in water (50 mL) for 1 hour. The resulting suspension was filtered and the solid washed with water and air dried to afford 3.8 g of the crude product as a white solid. The crude product was purified by flash chromatography (methylene chloride:ethyl acetate 8:2) to afford 2.3 g (50%) of N-benzyloxycarbonyl-α-amino-α-methylglutarimide as a white solid: mp 150.5–152.5° C.; ^1H NMR ($CDCl_3$) δ 8.21 (s, 1H), 7.34 (s, 5H), 5.59 (s, 1H), 5.08 (s, 2H), 2.74–2.57 (m, 3H), 2.28–2.25 (m, 1H), 1.54 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.06, 171.56, 154.68, 135.88, 128.06, 127.69, 127.65, 66.15, 54.79, 29.14, 28.70, 21.98; HPLC: Waters Nova-Pak C18 column, 4 micron, 3.9×150 mm, 1 mL/min, 240 nm, 20/80 CH_3CN/0.1% H_3PO_4(aq), 7.56 min (100%), Anal. Calcd For $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.88; H, 5.72; N, 10.07.

In a similar fashion from N-benzyloxycarbonyl-α-amino-α-ethylisoglutamine and N-benzyloxycarbonyl-α-amino-α-propylisoglutamine there is obtained N-benzyloxy-carbonyl-α-amino-α-ethylglutarimide and N-benzyloxycarbonyl-α-amino-α-propylglutarimide, respectively.

EXAMPLE 8

α-Amino-α-methylglutarimide hydrochloride

N-Benzyloxycarbonyl-α-amino-α-methylglutarimide (2.3 g, 8.3 mmol) was dissolved in ethanol (200 mL) with gentle heat and the resulting solution allowed to cool to room temperature. To this solution was added 4N hydrochloric acid (3 mL) followed by 10% Pd/C (0.4 g). The mixture was hydrogenated in a Parr apparatus under 50 psi of hydrogen for 3 hours. To the mixture was added water (50 mL) to dissolve the product. This mixture was filtered through a Celite pad which was washed with water (50 mL). The filtrate was concentrated in vacuo to afford a solid residue. The solid was slurried in ethanol (20 mL) for 30 min. The slurry was filtered to afford 1.38 g (93%) of α-amino-α-methylglutarimide hydrochloride as a white solid: ^1H NMR (DMSO-d_6) δ 11.25 (s, 1H), 8.92 (s, 3H), 2.84–2.51 (m, 2H), 2.35–2.09 (m, 2H), 1.53 (s, 3H); HPLC, Waters Nova-Pak C_{18} column, 4 micron, 1 mL/min, 240 nm, 20/80 CH_3CN/0.1% H_3PO_4(aq), 1.03 min (94.6%).

In a similar fashion from N-benzyloxycarbonyl-α-amino-α-ethylglutarimide and N-benzyloxycarbonyl-α-amino-α-propylglutarimide there is obtained α-amino-α-ethylglutarimide hydrochloride and α-amino-α-propylglutarimide hydrochloride, respectively.

EXAMPLE 9

3-(3-Nitrophthalimido)-3-methylpiperidine-2,6-dione

A stirred mixture of α-amino-α-methylglutarimide hydrochloride (1.2 g, 6.7 mmol), 3-nitrophthalic anhydride (1.3 g, 6.7 mmol), and sodium acetate (0.6 g, 7.4 mmol) in acetic acid (30 mL) was heated to reflux under nitrogen for 6 hours. The mixture then was cooled and concentrated in vacuo. The resulting solid was slurried in water (30 mL) and methylene chloride (30 ml) for 30 min. The suspension was filtered, the solid was washed with methylene chloride, and dried in vacuo (60° C., <1 mm) to afford 1.44 g (68%) of 3-(3-nitrophthalimido)-3-methylpiperidine-2,6-dione as a off-white solid: mp 265–266.5° C.; ^1H NMR (DMSO-d_6) δ 11.05 (s, 1H), 8.31 (dd, J=1.1 and 7.9 Hz, 1H), 8.16–8.03 (m, 2H), 2.67–2.49 (m, 3H), 2.08–2.02 (m, 1H) (s, 3H); ^{13}C NMR (DMSO-d_6) δ 172.20, 171.71, 165.89, 163.30, 144.19, 136.43, 133.04, 128.49, 126.77, 122.25, 59.22, 28.87, 28.49, 21.04; HPLC, Water Nova-Pak/C_{18} column, 4 micron, 1 mL/min, 240 nm, 20/80 CH_3CN/0.1% H_3PO_4(aq), 7.38 min(98%). Anal. Calcd For $C_{14}HB_{11}N_3O_6$: C, 53.00; H, 3.49; N, 13.24. Found: C, 52.77; H, 3.29; N, 13.00.

In a similar fashion from α-amino-α-ethylglutarimide hydrochloride and α-amino-α-propylglutarimide hydrochloride there is obtained 3-(3-nitrophthalimido)-3-ethylpiperidine-2,6-dione and 3-(3-nitrophthalimido)-3-propylpiperidine-2,6-dione, respectively.

EXAMPLE 10

3-(3-Aminophthalimido)-3-methylpiperidine-2,6-dione

3-(3-Nitrophthalimido)-3-methylpiperidine-2,6-dione (0.5 g, 1.57 mmol) was dissolved in acetone (250 mL) with gentle heat and then cooled to room temperature. To this solution was added 10% Pd/C (0.1 g) under nitrogen. The mixture was hydrogenated in a Parr apparatus at 50 psi of hydrogen for 4 hours. The mixture then was filtered through Celite and the pad washed with acetone (50 mL). The filtrate was concentrated in vacuo to yield a yellow solid. The solid was slurried in ethyl acetate (10 mL) for 30 minutes. The slurry then was filtered and dried (60° C., <1 mm) to afford 0.37 g (82%) of 3-(3-aminophthalimido)-3-methylpiperidine-2,6-dione as a yellow solid: mp 268–269° C.; ^{1}NMR (DMSO-d_6) δ 10.98 (s, 1H), 7.44 (dd, J=7.1 and

7.3 Hz, 1H), 6.99 (d, J=8.4 Hz, 1H), 6.94 (d, J=6.9 Hz, 1H), 6.52 (s, 2H), 2.71–2.47 (m, 3H), 2.08–1.99 (m, 1H), 1.87 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 172.48, 172.18, 169.51, 168.06, 146.55, 135.38, 131.80, 121.51, 110.56, 108.30, 58.29, 29.25, 28.63, 21.00; HPLC, Water Nova-Pak/C_{18} column, 4 micron, 1 mL/min, 240 nm, 20/80 CH_3CN/0.1%H_3PO_4(aq), 5.62 min (99.18%). Anal. Calcd For $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.60; H, 4.41; N, 14.36.

In a similar fashion from 3-(3-nitrophthalimido)-3-ethylpiperidine-2,6-dione and 3-(3-nitrophthalimido)-3-propylpiperidine-2,6-dione there is obtained 3-(3-aminophthalimido)-3-ethylpiperidine-2,6-dione and 3-(3-aminophthalimido)-3-propylpiperidine-2,6-dione, respectively.

EXAMPLE 11

Methyl 2-bromomethyl-3-nitrobenzoate

A stirred mixture of methyl 2-methyl-3-nitrobenzoate (17.6 g, 87.1 mmol) and N-bromosuccinimide (18.9 g, 105 mmol) in carbon tetrachloride (243 mL) was heated under gentle reflux with a 100 W light bulb situated 2 cm away shining on the reaction mixture overnight. After 18 hours, the reaction mixture was cooled to room temperature and filtered. The filtrate was washed with water (2×120 mL), brine(120 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to give a yellow solid. The product was purified by flash chromatography (hexane:ethyl acetate 8:2) to give 22 g (93%) of methyl 2-bromomethyl-3-nitrobenzoate as a yellow solid: mp 69–72° C.; ^{1}H NMR ($CDCl_3$) δ 8.13–8.09 (dd, J=1.36 and 7.86 Hz, 1H), 7.98–7.93 (dd, J=1.32 and 8.13 Hz, 1H), 7.57–7.51 (t, J=7.97Hz, 1H), 5.16 (s, 2H), 4.0 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 65.84, 150.56, 134.68, 132.64, 132.36, 129.09, 53.05, 22.70; HPLC: Waters Nova-Pak C_{18} column, 4 micron, 1 mL/min, 240 nm, 40/60 CH_3CN/0.1%H_3PO_4(aq), 8.2 min 99%. Anal. Calcd for $C_9H_8NO_4Br$: C, 39.44; H, 2.94; N, 5.11, Br, 29.15. Found: C, 39.51; H, 2.79; N, 5.02; Br, 29.32.

EXAMPLE 12

3-(1-Oxo4-nitroisoindolin-1-yl)-3-methylpiperidine2,6-dione

To a stirred mixture of α-amino-α-methylglutarimide hydrochloride (2.5 g, 14.0 mmol) and methyl 2-bromomethyl-3-nitrobenzoate(3.87 g, 14.0 mmol in dimethylformamide (40 mL) was added triethylamine (3.14 g, 30.8 mmol). The resulting mixture was heated to reflux under nitrogen for 6 hours. The mixture was cooled and then concentrated in vacuo. The resulting solid was slurried in water (50 mL) and CH_2Cl_2 for 30 min. The slurry was filtered, the solid washed with methylene chloride, and dried in vacuo (60° C., <1 mm) to afford 2.68 g (63%) of 3-(1-oxo-4-nitroisoindolin-1-yl)-3-methylpiperidine-2,6-dione as a off-white solid: mp 233–235° C; ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H), 8.49–8.46 (d, J=8.15 Hz, 1H), 8.13–8.09 (d, J=7.43 Hz, 1H), 7.86–7.79 (t, J=7.83 Hz, 1H), 5.22–5.0 (dd, J=19.35 and 34.6 Hz, 2H), 2.77–2.49 (m, 3H), 2.0–1.94 (m, 1H), 1.74 (S, 3H); ^{13}C NMR (DMSO-d_6) δ 173.07, 172.27, 164.95, 143.15, 137.36, 135.19, 130.11, 129.32, 126.93, 126.93, 57.57, 48.69, 28.9, 27.66, CH_2CN/0.1%H_3PO_4(aq), 4.54 min 99.6%. Anal. Calcd for $C_{14}H_{13}N_3O_5$: C, 55.45 ; H, 4.32; N, 13.86. Found: C, 52.16; H, 4.59; N, 12.47.

By substituting equivalent amounts of α-amino-α-ethylglutarimide hydrochloride and α-amino-α-propylglutarimide hydrochloride for α-amino-α-methylglutarimide hydrochloride, there is obtained respectively 3-(1-oxo-4-nitroisoindolin-1-yl)-3-ethylpiperidine-2,6-dione and 3-(1-oxo-4-nitroisoindolin-1-yl)-3-propylpiperidine-2,6-dione.

EXAMPLE 13

3-(1-Oxo-4-aminoisoindolin-1-yl)-3-methylpiperidine-2,6-dione

3-(1-Oxo-4-nitroisoindolin-1-yl)-3-methylpiperidine-2,6-dione (1.0 g, 3.3 mmol) was dissolved in methanol (500 mL) with gentle heat and allowed to cool to room temperature. To this solution was added 10% Pd/C (0.3 g) under nitrogen. The mixture was hydrogenated in a Parr apparatus at 50 psi of hydrogen for 4 hours. The mixture was filtered through Celite and the Celite washed with methanol (50 mL). The filtrate was concentrated in vacuo to an off white solid. The solid was slurried in methylene chloride (20 mL) for 30 min. The slurry was then filtered and the solid dried (60° C., <1 mm) to afford 0.54 g (60%) of 3-(1-oxo-4-aminoisoindolin-1-yl)-3-methylpiperidine-2,6-dione as a white solid: mp 268–270° C.; ^{1}H NMR (DMSO-d_6) δ 10.85 (s, 1H), 7.19–7.13 (t, J=7.63 Hz, 1H), 6.83–6.76 (m, 2H), 5.44 (s, 2H), 4.41(s, 2H), 2.71–2.49 (m, 3H), 1.9–1.8 (m, 1H), 1.67 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 173.7, 172.49, 168.0, 143.5, 132.88, 128.78, 125.62, 116.12, 109.92, 56.98, 46.22, 29.04, 27.77, 20.82; HPLC, Waters Nova-Pak/C18 column, 4 micron, 1 mL/min, 240 nm, 20/80 CH_3CN/0.1%H_3PO_4 (aq), 1.5 min (99.6%); Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38. Found: C, 58.99; H, 5.48; N, 14.29.

From 3-(1-oxo-4-nitroisoindolin-1-yl)-3-ethylpiperidine-2,6-dione and 3-(1-oxo-4-nitroisoindolin-1-yl)-3-propylpiperidine-2,6-dione there is similarly obtained 3-(1-oxo-4-aminoisoindolin-1-yl)-3-ethylpiperidine-2,6-dione and 3-(1-oxo-4-aminoisoindolin-1-yl)-3-propylpiperidine-2,6-dione, respectively.

EXAMPLE 14

S-4-Amino-2-(2,6-dioxopiperid-3-yl)isoindoline-1,3-dione

A. 4-Nitro-N-ethoxycarbonylphthalimide

Ethyl chloroformate (1.89 g, 19.7 mmol) was added dropwise over 10 min to a stirred solution of 3-nitrophthalimide (3.0 g, 15.6 mmol) and triethylamine (1.78 g, 17.6 mmol) in dimethylformamide (20 mL) at 0–5° C. under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 4 hours. The mixture was then slowly added to an agitated mixture of ice and water (60 mL). The resulting slurry was filtered and the solid was crystallized from chloroform (15 mL) and pet ether (15 mL) to afford 3.1 g (75%) of the product as an off-white solid: mp 100–100.5° C.; ^{1}H NMR ($CDCl_3$) δ 8.25(d, J=7.5 Hz, 1H), 8.20(d, J=8.0 Hz, 1H), 8.03(t, J=7.9 Hz, 1H), 4.49(q, J=7.1 Hz, 2H), 1.44(t, J=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.45, 158.40, 147.52, 145.65, 136.60, 132.93, 129.65, 128.01, 122.54, 64.64, 13.92; HPLC, Waters Nova-Pak/C18, 3.9×150 mm, 4 micron, 1 mL/min, 240 nm, 30/70 CH_3CN/0.1%H_3PO_4(aq), 5.17 min(98.11%); Anal. Calcd for $C_{11}H_8N_2O_6$: C, 50.00; H, 3.05; N, 10.60. Found: C, 50.13; H, 2.96; N, 10.54.

B. t-Butyl N-(4-nitrophthaloyl)-L-glutamine

A stirred mixture of 4-nitro-N-ethoxycarbonylphthalimide (1.0 g, 3.8 mmol), L-glutamine t-butyl ester hydrochloride (0.90 g, 3.8 mmol) and triethylamine (0.54 g, 5.3 mmol) in tetrahydrofuran (30 mL) was

heated to reflux for 24 hours. The tetrahydrofuran was removed in vacuo and the residue was dissolved in methylene chloride (50 mL). The methylene chloride solution was washed with water (2×15 mL), brine (15 mL) and then dried (sodium sulfate). The solvent was removed in vacuo and the residue was purified by flash chromatograph (7:3 methylene chloride:ethyl acetate) to give 0.9 g (63%) of a glassy material: ^{1}H NMR ($CDCl_3$) δ 8.15(d, J=7.9 Hz, 2H), 7.94(t, J=7.8 Hz, 1H), 5.57(b, 2H), 4.84(dd, J=5.1 and 9.7 Hz, 1H), 2.53–2.30(m, 4H), 1.43(s, 9H); HPLC, Waters Nova-Pak/C18, 3.9×150 mm, 4 micron, 1 mL/min, 240 nm, 30/70 CH_3CN/0.1%H_3PO_4(aq), 6.48 min(99.68%); Chiral Analysis, Daicel Chiral Pak AD, 0.4×25 Cm, 1 mL/min, 240 nm, 5.32 min(99.39%); Anal. Calcd for $C_{17}H_{19}N_3O_7$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.21; H, 5.08; N, 10.85.

C. N-(4-Nitrophthaloyl)-L-glutamine

Hydrogen chloride gas was bubbled into a stirred 5° C. solution of t-butyl N-(4-nitrophthaloyl)-L-glutamine (5.7 g, 15.1 mmol) in methylene chloride (100 mL) for 25 min. The mixture was then stirred at room temperature for 16 hours. Ether (50 mL) was added and the resulting mixture was stirred for 30 min. The resulting slurry was filtered to yield 4.5 g of crude product as a solid, which was used directly in the next reaction: ^{1}H NMR (DMSO-d_6) δ 8.36(dd, J=0.8 and 8.0 Hz, 1H), 8.24(dd, J=0.8 and 7.5 Hz, 1H), 8.11(t, J=7.9 Hz, 1H), 7.19(b, 1H), 6.72(b, 1H), 4.80(dd, J=3.5 and 8.8 Hz, 1H), 2.30–2.10(m, 4H).

D. (S)-2-(2,6-dioxo(3-piperidyl3)-4-nitroisoindoline-1,3-dione

A stirred suspension of N-(4-nitrophthaloyl)-L-glutamine (4.3 g, 13.4 mmol) in anhydrous methylene chloride (170 mL) was cooled to −40° C. (IPA/dry ice bath). Thionyl chloride (1.03 mL, 14.5 mmol) was added dropwise to the mixture followed by pyridine (1.17 mL, 14.5 mmol). After 30 minutes, triethylamine (2.06 mL, 14.8 mmol) was added and the mixture was stirred at −30 to −40° C. for 3 hours. The mixture was allowed to warm to room temperature, filtered and washed with methylene chloride to afford 2.3 g, (57%) of the crude product. Recrystallization from acetone (300 mL) afforded 2 g of the product as a white solid: mp 259.0– 284.0° C.(dec.); ^{1}H NMR (DMSO-d_6) δ 11.19(s, 1H), 8.34(d, J=7.8 Hz, 1H), 8.23(d, J=7.1 Hz, 1H), 8.12(t, J=7.8 Hz, 1H), 5.25–5.17(dd, J=5.2 and 12.7 Hz, 1H), 2.97–2.82(m, 1H), 2.64–2.44(m, 2H), 2.08–2.05(m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.67, 169.46, 165.15, 162.50, 144.42, 136.78, 132.99, 128.84, 127.27, 122.53, 49.41, 30.84, 21.71; HPLC, Waters Nova-Pak/C18, 3.9×150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH_3CN/0.1%H_3PO_4(aq) 4.27 min(99.63%); Anal. Calcd for $C_{13}H_9N_3O_6$: C, 51.49; H, 2.99; N, 13.86. Found: C, 51.67; H, 2.93; N, 13.57.

E. S-4-Amino-2-(2, 6-dioxopiperid-3-yl)isoindoline-1,3-dione

A mixture of (S)-3-(4'-nitrophthalimido)piperidine-2,6-dione (0.76 g, 2.5 mmol) and 10%Pd/C (0.3 g) in acetone (200 mL) was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 24 hours. The mixture was filtered through celite and the filtrate was concentrated in vacuo. The solid residue was slurried in hot ethyl acetate for 30 min and filtered to yield 0.47 g (69%) of the product as a yellow solid: mp 309–310° C.; ^{1}HNMR (DMSO-d_6) δ 11.10 (s, 1H), 7.47(dd, J=7.2 and 8.3 Hz, 1H), 7.04–6.99(dd, J=6.9 and 8.3 Hz, 2H). 6.53(s, 2H), 5.09–5.02(dd, J=5.3 and 12.4 Hz, 1H), 2.96–2.82(m, 1H), 2.62–2.46(m, 2H), 2.09–1 99 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.80, 170.10, 168.57, 167.36, 146.71, 135.44, 131.98, 121.69, 110.98, 108.54, 48.48, 30.97, 22.15; HPLC, Waters Nova-Pak/C18, 3.9×150 mm, 4 micron, 1 mL/min. 240 nm, 15/85 CH_3CN/ 0.1%H_3PO_4(aq) 4.99 min(98.77%); Chiral analysis, Daicel Chiral Pak AD, 0.46×25 cm, 1 mL/min, 240 nm, 30/70 Hexane/IPA 9.55 min (1.32%). 12.55 min (97.66%); Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.15; H,4.15; N, 14.99.

EXAMPLE 15

R-4-Amino-2-(2,6-dioxopiperid-3-yl))isoindoline-1, 3-dione

A. t-Butyl N-(4-nitrophthaloyl)-D-glutamine

A stirred mixture of 4-nitro-N-ethoxycarbonylphthalimide (5.9 g, 22.3 mmol), D-glutamine t-butyl ester (4.5 g, 22.3 mmol) and triethylamine (0.9 g, 8.9 mmol) in tetrahydrofuran (100 mL) was refluxed for 24 hours. The mixture was diluted with methylene chloride (100 mL) and washed with water (2×50 mL), brine (50 mL) and then dried. The solvent was removed in vacuo and the residue was purified by flash chromatography (2% CH_3OH in methylene chloride) to afford 6.26 g (75%) of the product as a glassy material: ^{1}H NMR ($CDCl_3$) δ 8.12(d, J=7.5 Hz, 2H), 7.94 (dd, J=7.9 and 9.1 Hz, 1H), 5.50(b, 1H), 5.41(b, 1H), 4.85(dd, J=5.1 and 9.8 Hz, 1H), 2.61–2.50(m, 2H), 2.35–2.27(m,2H), 1.44(s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.77, 167.06, 165.25, 162.51, 145.07, 135.56, 133.78, 128.72, 127.27, 123.45, 83.23, 53.18, 32.27, 27.79, 24.42,; HPLC, Waters Nova-Pak/C18, 3.9×150 mm, 4 micron, 1 mL/min, 240 nm, 25/75 CH_3CN/0.1%H_3PO_4(aq) 4.32 min(99.74%); Chiral analysis, Daicel Chiral Pak AD, 0.46×25 cm, 1 mL/min, 240 nm, 55/45 Hexane/IPA 5.88 min(99.68%); Anal. Calcd for $C_{17}H_{19}N_3O_7$: C, 54.11; H, 5.08; N, 11.14. Found: C, 54.25; H, 5.12; N, 10.85.

B. N-(4-Nitrophthaloyl)-D-glutamine

Hydrogen chloride gas was bubbled into a stirred 5° C. solution of t-butyl N-(4-nitrophthaloyl)-D-glutamine (5.9 g, 15.6 mmol) in methylene chloride (100 mL) for 1 hour then stirred at room temperature for another hour. Ether (100 mL) was added and stirred for another 30 minutes. The mixture was filtered, the solid was washed with ether (60 mL) and dried (40° C., <1 mm Hg) to afford 4.7 g (94%) of the product: ^{1}H NMR (DMSO-d_6) δ 8.33(d, J=7.8 Hz, 1H), 8.22(d, J=7.2 Hz, 1H), 8.11(t, J=7.8 Hz, 1H), 7.19(b, 1H), 6.72(b, 1H), 4.81(dd, J=4.6 and 9.7 Hz, 1H), 2.39–2.12(m, 4H); ^{13}C NMR (DMSO-d_6) δ 173.21. 169.99, 165.41, 162.73, 144.45, 136.68, 132.98, 128.80, 127.23, 122.52, 51.87, 31.31, 23.87.

C. (R)-2-(2, 6-dioxo(3-piperidyl))-4-nitroisoindoline-1,3-dione

A stirred suspension of N-(4'-nitrophthaloyl)-D-glutamine (4.3 g, 13.4 mmol) in anhydrous methylene chloride (170 mL) was cooled to −40° C. with isopropanol/dry ice bath. Thionyl chloride (1.7 g, 14.5 mmol) was added dropwise followed by pyridine (1.2 g, 14.5 mmol). After 30 min, triethylamine (1.5 g, 14.8 mmol) was added and the mixture was stirred at −30 to −40° C. for 3 hours. The mixture was filtered, the solid washed with methylene chloride (50 mL) and dried (60° C., <1 mm Hg) to give 2.93 g of the product. Another 0.6 g of the product was obtained from the methylene chloride filtrate. Both fractions were combined (3.53 g) and recrystallized from acetone (450 mL) to afford 2.89 g (71%) of the product as a white solid: mp 256.5–257.5° C.; ^{1}H NMR (DMSO-d_6) δ 11.18(s, 1H), 8.34(dd, J=0.8 and 7.9 Hz, 1H), 8.23(dd, J=0.8 and 7.5 Hz, 1H), 8.12(t, J=7.8 Hz, 1H), 5.22(dd, J=5.3 and 12.8 Hz, 1H), 2.97–2.82(m, 1H), 2.64–2.47(m, 2H), 2.13–2.04(m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.66, 169.44, 165.14, 162.48, 144.41, 136.76, 132.98, 128.83, 127.25, 122.52, 49.41, 30.83, 21.70; HPLC, Waters Nova-Pak/C18, 3.9×150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH_3CN/0.1%H_3PO_4(aq) 3.35 min(100%); Anal. Calcd for $C_{13}H_9N_3O_6$: C, 51.49; H, 2.99; N, 13.86. Found: C, 51.55; H, 2.82; N, 13.48.

D. (R)-4-Amino-2-(2,6-dioxopiperid-3-yl)isoindoline-1,3-dione

A mixture of R-3-(4'-nitrophthalimido)-piperidine-2,6-dione (1.0 g, 3.3 mmol) and 10% Pd/C (0.2 g) in acetone (250 mL) was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 4 hours. The mixture was filtered through celite and the filtrate was concentrated in vacuo. The resulting yellow solid was slurried in hot ethyl acetate (20 mL) for 30 min to give after filtration and drying 0.53 g (59%) of the product as a yellow solid: mp 307.5–309.5° C.; ^{1}H NMR (DMSO-d_6) δ 11.06(s, 1H), 7.47(dd, J=7.0 and 8.4 Hz, 1H), 7.02(dd, J=4.6 and 8.4 Hz, 2H), 6.53(s, 2H), 5.07(dd, J=5.4 and 12.5 Hz, 1H), 2.95–2.84(m, 1H), 2.62–2.46(m, 2H), 2.09–1.99 (m, 1H); ^{13}C NMR(DMSO-d_6) δ 172.78, 170.08, 168.56, 167.35, 146.70, 135.43, 131.98, 121.68, 110.95, 108.53, 48.47, 30.96, 22.14; HPLC, Waters Nove-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH_3CN/0.1%H_3PO_4(aq) 3.67 min(99.68%); Chiral analysis, Daicel Chiral Pak AD, 0.46x25 cm, 1 mL/min, 240 nm, 30/70 Hexane/IPA 7.88 min (97.48%); Anal. Calcd for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06; N, 15.38. Found: C, 57.34; H, 3.91; N, 15.14.

EXAMPLE 16

3-(4-Amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione

A.Methyl 2-bromomethyl-3-nitrobenzoate

A stirred mixture of methyl 2-methyl-3-nitrobenzoate (14.0 g, 71.7 mmol) and N-bromosuccinimide (15.3 g, 86.1 mmol) in carbon tetrachloride (200 mL) was heated under gentle reflux for 15 hours while a 100W bulb situated 2 cm away was shining on the flask. The mixture was filtered and the solid was washed with methylene chloride (50 mL). The filtrate was washed with water (2x100 mL), brine (100 mL) and dried the solvent was removed in vacuo and the residue was purified by flash chromatography (hexane/ethyl acetate, 8/2) to afford 19 g (96%) of the product as a yellow solid: mp 70.0–71.53° C.; ^{1}H NMR ($CDCl_3$) δ 8.12–8.09(dd, J=1.3 and 7.8 Hz, 1H), 7.97–7.94(dd, J=1.3 and 8.2 Hz, 1H), 7.54(t, J=8.0 Hz, 1H), 5.15(s, 2H), 4.00(s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.85, 150.58, 134.68, 132.38, 129.08, 127.80, 53.06, 22.69; HPLC. Water Nove-Pak/ C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 40/60 CH_3CN/0.1%H_3PO_4(aq) 7.27 min(98.92%); Anal. Calcd for $C_9H_8NO_4Br$: C, 39.44; H, 2.94; N, 5.11; Br, 29.15. Found:C, 39.46; H, 3.00; N, 5.00; Br, 29.11.

B. t-Butyl N-(1-oxo-4-nitroisoindolin-2-yl)-L-glutamine

Triethylamine (2.9 g, 28.6 mmol) was added dropwise to a stirred mixture of methyl 2-bromomethyl-3-nitrobenzoate (3.5 g, 13.0 mmol) and L-glutamine t-butyl ester hydrochloride (3.1 g, 13.0 mmol) in tetrahydrofuran (90 mL). The mixture was heated to reflux for 24 hours. To the cooled mixture was added methylene chloride (150 mL) and the mixture was washed with water (2x40 mL), brine (40 mL) and dried. The solvent was removed in vacuo and the residue was purified by flash chromatography (3% CH_3OH in methylene chloride) to afford 2.84 g (60%) of crude product which was used directly in the next reaction: ^{1}H NMR ($CDCl_3$) δ 8.40(d, J=8.1 Hz, 1H), 8.15(d, J=7.5 Hz, 1H), 7.71(t, J=7.8 Hz, 1H), 5.83(s, 1H), 5.61(s, 1H), 5.12(d, J=19.4 Hz, 1H), 5.04–4.98(m,1H), 4.92(d, J=19.4 Hz, 1H), 2.49–2.22(m, 4H), 1.46(s, 9H); HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 25/75 CH_3CN/0.1%H_3PO4(aq) 6.75 min(99.94%).

C. N-(1-Oxo-4-nitroisoindolin-2-yl)-L-glutamine

Hydrogen chloride gas was bubbled into a stirred 5° C. solution of t-butyl N-(1-oxo4-nitro-isoindolin-2-yl)-L-glutamine (3.6 g, 9.9 mmol) in methylene chloride (60 mL) for 1 hour. The mixture was then stirred at room temperature for another hour. Ether (40 mL) was added and the resulting mixture was stirred for 30 minutes. The slurry was filtered, washed with ether and dried to afford 3.3 g of the product: ^{1}H NMR (DMSO-d_6) δ 8.45(d, J=8.1 Hz, 1H), 8.15(d, J=7.5 Hz, 1H), 7.83(t, J=7.9 Hz, 1H), 7.24(s, 1H), 6.76(s, 1H), 4.93(s, 2H), 4.84–4.78(dd, J=4.8 and 10.4 Hz, 1H), 2.34–2.10(m, 4H); ^{13}C NMR (DMSO-d_6) δ 173.03, 171.88, 165.96, 143.35, 137.49, 134.77, 130.10, 129.61, 126.95, 53.65, 48.13, 31.50, 24.69; Anal. Calcd for $C_{13}H_{13}N_3O_6$: C, 50.82; H, 4.26; N, 13.68. Found: C, 50.53; H, 4.37; N, 13.22.

D. (S)-3-(1-Oxo-4-nitroisoindolin-2-yl)piperidine-2,6-dione

A stirred suspension mixture of N-(1-oxo-4-nitroisoindolin-2-yl)-L-glutamine (3.2 g, 10.5 mmol) in anhydrous methylene chloride (150 mL) was cooled to −40° C. with isopropanol/dry ice bath. Thionyl chloride (0.82 mL, 11.3 mmol) was added dropwise to the cooled mixture followed by pyridine (0.9 g 11.3 mmol). After 30 min, triethylamine (1.2 g, 11.5 mmol) was added and the mixture was stirred at −30 to −40° C. for 3 hours. The mixture was poured into ice water (200 mL) and the aqueous layer was extracted with methylene chloride (40 mL). The methylene chloride solution was washed with water (2x60 mL), brine (60 mL) and dried. The solvent was removed in vacuo and the solid residue was slurried with ethyl acetate (20 mL) to give 2.2 g (75%) of the product as a white solid: mp 285° C.; ^{1}H NMR (DMSO-d_6) δ 11.04(s, 1H), 8.49–8.45(dd, J=0.8 and 8.2 Hz, 1H), 8.21–8.17(dd, J=7.3 Hz, 1H), 7.84(t, J=7.6 Hz, 1H), 5.23–5.15(dd, J=4.9 and 13.0 Hz, 1H), 4.96(dd, J=19.3 and 32.4 Hz, 2H), 3.00–2.85(m, 1H), 2.64–2.49(m, 2H), 2.08–1.98(m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.79, 170.69, 165.93, 143.33, 137.40, 134.68, 130.15, 129 60, 127.02, 51.82, 48.43, 31.16. 22.23; HPLC, Waters Nove-Pak/C18, 3.9x150 mm, 4 micron, I mL/min, 240 nm, 20/80 CH_3CN/0.1%H_3PO_4(aq) 3.67 min(100%); Anal. Calcd for $C_{13}H_{11}N_3O_5$: C, 53.98; H, 3.83; N, 14.53. Found: C, 53.92; H, 3.70; N, 14.10.

E. (S)-3-(1-Oxo-4-aminoisoindolin-2-yl)piperidine-2,6 dione

A mixture of (S)-3-(1-oxo-4-nitroisoindolin-2-yl) piperidine-2,6-dione (1.0 g, 3.5 mmol) and 10% Pd/c (0.3 g) in methanol (600 mL) was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 5 hours. The mixture was filtered through Celite and the filtrate was concentrated in vacuo. The solid was slurried in hot ethyl acetate for 30 min, filtered and dried to afford 0.46 g (51%) of the product as a white solid: mp 235.5–239° C.; ^{1}H NMR (DMSO-d_6) δ 11.01(s, 1H), 7.19(t, J=7.6 Hz, 1H), 6.90(d, J=7.3 Hz, 1H), 6.78(d, J=7.8 Hz, 1H), 5.42(s, 2H), 5.12(dd, J=5.1 and 13.1 Hz, 1H), 4.17(dd, J=17.0 and 28.8 Hz, 2H), 2.92–2.85(m, 1H), 2.64–2.49(m, 1H), 2.34–2.27(m, 1H),2.06–1.99(m, 1H); ^{13}C NMR (DMSO-d_6) δ 172.85, 171.19, 168.84, 143.58, 132.22, 128.79, 125.56, 116.37, 110.39, 51.48, 45.49, 31.20, 22.74; HPLC, Waters Nova-Pak/C18, 3.9x150 mm, 4 micron, 1 mL/min, 240 nm, 10/90 CH_3CN/0.1%H_3PO_4(aq) 0.96 min(100%); Chiral analysis, Daicel Chiral Pak AD, 40/60 Hexane/IPA, 6.60 min(99.42%); Anal. Calcd for $C_{13}H_{13}N_3O_3$: C, 60.23; H, 5.05; N, 16.21. Found: C, 59.96; H, 4.98; N, 15.84.

EXAMPLE 17

3-(4-Amino-1-oxoisoindolin-2yl)-3-methylpiperidine-2,6-dione

A. N-Benzyloxycarbonyl-3-amino-3-methylpiperidine-2,6-dione

A stirred mixture of N-benzyloxycarbonyl-α-methyl-isoglutamine (11.3 g, 38.5 mmol), 1,1'-carbonyldiimidazole (6.84 g, 42.2 mmol) and 4-dimethylaminopyridine (0 05 g) in tetrahydrofuran (125 ml) was heated to reflux under nitrogen for 19 hours. The reaction mixture was concentrated in vacuo to an oil. The oil was slurried in water (50

mL) for 1 hour then filtered, washed with water, air dried to afford 7.15 g of white solid. The crude product was purified by flash chromatography (2:8 ethyl acetate:methylene chloride) to afford 6.7 g (63%) of the product as a white solid: mp 151–152° C.; ^{1}H NMR ($CDCl_3$) δ 8.24 (s, 1H), 7.35 (s, 5H), 5.6 (s, 1H). 5.09 (s, 2H), 2.82–2.53 (m, 3H), 2.33–2.26 (m, 1H), 1.56 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.4, 172.4, 154.8, 136.9, 128.3, 127.8, 127.7, 65.3, 54.6, 29.2, 29.0, 22.18; HPLC: Waters Nova-Pak/C_{18} column, 4 micron, 3.9x150 mm, 1 ml/min, 240 nm, 20/80 CH_3CN/H_3PO_4(aq), 6.6 min, 100%). Anal. Calcd for $C_{14}H_{14}N_2O_4$. Theory: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.94; H, 5.76; N, 10.10.

B. 3-Amino-3-methylpiperidine-2,6-dione

N-benzyloxycarbonyl-3-amino-3-methylpiperidine-2,6-dione (3.0 g, 10.9 mmol) was dissolved in ethanol (270 mL) with gentle heat and then cooled to room temperature. To this solution was added 4 N HCl (7 mL) followed by 10% Pd/C (0.52 g). The mixture was hydrogenated under 50 psi of hydrogen for 3 hours. To the mixture was then added water (65 mL) to dissolve the product. The mixture was filtered through a celite pad and the celite pad washed with water (100 mL). The filtrate was concentrated in vacuo to a solid residue. This solid was slurried in ethanol (50 mL) for 30 min. The slurry was filtered to afford 3.65 g (94%) of the product as a white solid: ^{1}H NMR (DMSO-d_6) δ 11.25 (s, 1H), 8.9 (s, 3H), 2.87–2.57 (m, 2H), 2.35–2.08 (m, 2H), 1.54 (s, 3H); HPLC (Waters Nova-Pak/C_{18} column, 4 micron, 1 ml/min. 240 nm, 15/85 CH_3CN/H_3PO_4(aq), 1.07 min, 100%).

C. 3-Methyl-3-(4-nitro-1-oxoisoindolin-2-yl)piperidine-2,6-dione

To a stirred mixture of α-amino-α-methyl-glutarimide hydrochloride (2.5 g, 14.0 mmol) and methyl 2-bromomethyl-3-nitro benzoate (3.87 g, 14 mmol) in dimethylformamide (40 mL) was added triethylamine (3.14 g, 30.8 mmol) under nitrogen. The mixture was heated to reflux for 6 hours. The mixture was cooled and then concentrated in vacuo. The solid residue was slurried in water (50 mL) and methylene chloride for 30 min. The slurry was filtered and the solid washed with methylene chloride and dried (60° C., <1 mm). Recrystallization from methanol (80 mL) yielded 0.63 g (15%) of the product as an off white solid: mp 195–197° C.; ^{1}H NMR (DMSO-d_6) δ 10.95 (s, 1H), 8.49–8.46 (d, J=8.2 Hz, 1H), 8.13–8.09 (d, J=7.4 Hz, 1H), 7.86–7.79 (t, J=7.8 Hz, 1H), 5.22–5.0 (dd, J=19.4 and 34.6 Hz, 2H), 2.77–2.49 (m, 3H), 2.0–1.94 (m, 1H), 1.74 (S, 3H); ^{13}C NMR (DMSO-d_6) δ 173.1, 172.3, 165.0, 143.2, 137.4, 135.2, 130.1, 129.3, 126.9, 57.6, 48.7, 28.9, 27.7, 20.6; HPLC (Waters Nova-Pak/C_{18} is column, 4 micron, 1 ml/min, 240nm, 20/80 CH_3CN/H_3PO_4(aq), 4.54 min, 99.6%); Anal Calcd. For $C_{14}H_{13}N_3O_5$; C, 55.45; H, 4.32; N, 13.86. Found: C, 55.30; H, 4.48; N, 13.54.

D. 3-Methyl-3-(4-amino-1-oxoisoindolin-2yl)piperidine-2,6-dione

3-Methyl-3-(4-nitro-1-oxoisoindolin-2-yl)piperidine-2,6-dione (1.0 g, 3.3 mmol) was dissolved in methanol (500 mL) with gentle heat and then cooled to room temperature. To this solution was added 10% Pd/C (0.3 g) under nitrogen. The mixture was hydrogenated in a Parr-Shaker apparatus at 50 psi of hydrogen for 4 hours. The mixture was filtered through celite pad and the celite pad washed with methanol (50 mL). The filtrate was concentrated in vacuo to a off white solid. The solid was slurried in methylene chloride (20 mL) for 30 min. The slurry was filtered and the solid dried (60° C., <1 mm). The solid was to recrystallized from methanol (3 times, 100 ml /time) to yield 0.12 g (13.3%) of

the product as a white solid: mp 289–292° C.; ^{1}H NMR (DMSO-d_6) δ 10.85 (s, 1H), 7.19–7.13 (t, J=7.6 Hz, 1H), 6.83–6.76 (m, 2H), 5.44 (s, 2H), 4.41(s, 2H). 2.71–2.49 (m, 3H), 1.9–1.8 (m, 1H), 1.67 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 173.7, 172.5, 168.0, 143.5, 132.9, 128.8, 125.6, 116.1, 109.9, 57.0, 46.2, 29.0, 27.8, 20.8; HPLC (Waters Nova-Pak/C_{18} column, 4 micron, 1 ml/min, 240 nm, 20/80 CH_3CN/H_3PO_4(aq), 1.5 min. 99.6%); Anal. Calcd. For $C_{14}H_{15}N_3O_3$; C, 61.53; H, 5.53; N, 15.38. Found: C, 61.22; H, 5.63; N, 15.25.

EXAMPLE 18

Tablets, each containing 50 mg of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)	
1,3-dioxo-2-(2,6-dioxo piperidin-3-yl)-5-amino isoindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 19

Tablets, each containing 100 mg of 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)	
1,3-dioxo-2-(2,6-dioxo piperidin-3-yl)-5-amino isoindoline	100.0 g
lactose	100.0 g
wheat starch	47.0 g
magnesium stearate	3.0 g

All the solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to 100 mL of boiling water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 20

Tablets for chewing, each containing 75 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, can be prepared in the following manner:

Composition (for 1000 tablets)	
1-oxo-2-(2,6-dioxo-piperidin-3-yl)-4-amino-isoindoline	75.0 g
mannitol	230.0 g
lactose	150.0 g
talc	21.0 g
glycine	12.5 g
stearic acid	10.0 g
saccharin	1.5 g
5% gelatin solution	q.s.

All the solid ingredients are first forced through a sieve of 0.25 mm mesh width. The mannitol and the lactose are mixed, granulated with the addition of gelatin solution, forced through a sieve of 2 mm mesh width, dried at 50° C. and again forced through a sieve of 1.7 mm mesh width 1-Oxo-2-(2,6-dioxopiperidin-3-yl)4-aminoisoindoline, the glycine and the saccharin are carefully mixed, the mannitol, the lactose granulate, the stearic acid and the talc are added and the whole is mixed thoroughly and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking groove on the upper side.

EXAMPLE 21

Tablets, each containing 10 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-5-aminoisoindoline, can be prepared in the following manner:

Composition (for 1000 tablets)	
1-oxo-2-(2,6-dioxo-piperidin-3-yl)-5-amino-isoindoline	10.0 g
lactose	328.5 g
corn starch	17.5 g
polyethylene glycol 6000	5.0 g
talc	25.0 g
magnesium stearate	4.0 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. Then the active imide ingredient, lactose, talc, magnesium stearate and half of the starch are intimately mixed. The other half of the starch is suspended in 65 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 260 mL of water. The resulting paste is added to the pulverulent substances, and the whole is mixed and granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking notch on the upper side.

EXAMPLE 22

Gelatin dry-filled capsules, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline, can be prepared in the following manner:

Composition (for 1000 capsules)	
1-oxo-2-(2,6-dioxo-piperidin-3-yl)-6-amino-isoindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

The sodium lauryl sulfate is sieved into the 1-oxo-2-(2,6-dioxopiperidin-3-yl)-6-aminoisoindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 23

A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

1-oxo-2-(2,6-dioxo-piperidin-3-yl)-7-amino-isoindoline	5.0 g
sodium chloride	22.5 g
phosphate buffer pH 7.4	300.0 g
demineralized water	to 2500.0 mL

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-7-aminoisoindoline is dissolved in 1000 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

EXAMPLE 24

Tablets, each containing 50 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)	
1-oxo-2-(2,6-dioxo-piperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water. The resulting paste is added to the pulverulent substances and the mixture is

granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 25

Tablets, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrachloroisoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)	
1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrachloroisoindoline	100.0 g
lactose	300.0 g
wheat starch	47.0 g
magnesium stearate	3.0 g

All the solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to 100 mL of boiling water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 26

Tablets for chewing, each containing 75 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline, can be prepared in the following manner:

Composition (for 1000 tablets)	
1-oxo-2-(2,6-dioxo-piperidin-3-yl)-4,5,6,7-tetra-fluoroisoindoline	75.0 g
mannitol	230.0 g
lactose	150.0 g
talc	21.0 g
glycine	12.5 g
stearic acid	10.0 g
saccharin	1.5 g
5% gelatin solution	q.s.

All the solid ingredients are first forced through a sieve of 0.25 mm mesh width. The mannitol and the lactose are mixed, granulated with the addition of gelatin solution, forced through a sieve of 2 mm mesh width, dried at 50° C. and again forced through a sieve of 1.7 mm mesh width. 1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline, the glycine and the saccharin are carefully mixed, the mannitol, the lactose granulate, the stearic acid and the talc are added and the whole is mixed thoroughly and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking groove on the upper side.

EXAMPLE 27

Tablets, each containing 10 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethylisoindoline, can be prepared in the following manner:

Composition (for 1000 tablets)	
1-oxo-2-(2,6-dioxo-piperidin-3-yl)-4,5,6,7-tetramethylisoindoline	10.0 g
lactose	328.5 g
corn starch	17.5 g
polyethylene glycol 6000	5.0 g
talc	25.0 g
magnesium stearate	4.0 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. Then the active imide ingredient, lactose, talc, magnesium stearate and half of the starch are intimately mixed. The other half of the starch is suspended in 65 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 260 mL of water. The resulting paste is added to the pulverulent substances, and the whole is mixed and granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 10 mm diameter which are concave on bothsides and have a breaking notch on the upper side.

EXAMPLE 28

Gelatin dry-filled capsules, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisoindoline, can be prepared in the following manner:

Composition (for 1000 capsules)	
1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxy isoindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

The sodium lauryl sulfate is sieved into the 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetramethoxyisoindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 30

A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

1-oxo-2-(2,6-dioxopipendin-3-yl)-4,5,6,7-tetrafluoroisoindoline	5.0 g
sodium chloride	22.5 g
phosphate buffer pH 7.4	300.0 g
demineralized water	to 2500.0 mL

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline is dissolved in 1000 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

EXAMPLE 31

Tablets, each containing 50 mg of 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)	
1-oxo-2-(2,6-dioxo-3-methyl piperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 32

Tablets, each containing 100 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-aminoisoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)	
1-oxo-2-(2,6-dioxo piperidin-3-yl)-4-amino isoindoline	100.0 g
lactose	100.0 g
wheat starch	47.0 g
magnesium stearate	3.0 g

All the solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to 100 mL of boiling water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 33

Tablets for chewing, each containing 75 mg of 2-(2,6-dioxopiperidin-3-yl)-4-aminophthalimide, can be prepared in the following manner:

Composition (for 1000 tablets)	
2-(2,6-dioxo-3-methylpiperidin 3-yl)-4-aminophthalimide	75.0 g
mannitol	230.0 g
lactose	150.0 g
talc	21.0 g
glycine	12.5 g
stearic acid	10.0 g
saccharin	1.5 g
5% gelatin solution	q.s.

All the solid ingredients are first forced through a sieve of 0.25 mm mesh width. The mannitol and the lactose are mixed, granulated with the addition of gelatin solution, forced through a sieve of 2 mm mesh width, dried at 50° C. and again forced through a sieve of 1.7 mm mesh width. 2-(2,6-Dioxo-3-methylpiperidin-3-yl)-4-aminophthalimide, the glycine and the saccharin are carefully mixed, the mannitol, the lactose granulate, the stearic acid and the talc are added and the whole is mixed thoroughly and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking groove on the upper side.

EXAMPLE 34

Tablets, each containing 10 mg of 2-(2,6-dioxoethylpiperidin-3-yl)-4-aminophthalimide, can be prepared in the following manner:

Composition (for 1000 tablets)	
2-(2,6-dioxoethylpiperidin-3-yl)-4-aminophthalimide	10.0 g
lactose	328.5 g
corn starch	17.5 g
polyethylene glycol 6000	5.0 g
talc	25.0 g
magnesium stearate	4.0 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. Then the active imide ingredient, lactose, talc, magnesium stearate and half of the starch are intimately mixed. The other half of the starch is suspended in 65 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 260 mL of water. The resulting paste is added to the pulverulent substances, and the whole is mixed and granulated, if necessary with the addition of water. The granulate is dried overnight at 35° C., forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 10 mm diameter which are concave on both sides and have a breaking notch on the upper side.

EXAMPLE 35

Gelatin dry-filled capsules, each containing 100 m of 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline, can be prepared in the following manner:

Composition (for 1000 capsules)	
1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

The sodium lauryl sulfate is sieved into the 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 36

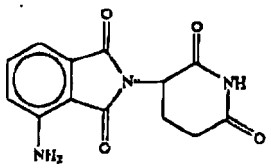
A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

1-oxo-2-(2,6-dioxo-3-methyl piperidin-3-yl)-4,5,6,7-tetrafluoro isoindoline	5.0 g
sodium chloride	22.5 g
phosphate buffer pH 7.4	300.0 g
demineralized water	to 2500.0 mL

1-Oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4,5,6,7-tetrafluoroisoindoline is dissolved in 1000 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

What is claimed is:

1. A method of treating inflammation, inflammatory disease or autoimmune disease in a mammal which comprises administering thereto an effective amount of a compound of the formula:



or an acid addition salt thereof.

2. The method according to claim 1 wherein said disease is rheumatoid arthritis.

3. The method according to claim 1 wherein said disease is osteoarthritis.

4. The method according to claim 1 wherein said disease is inflammatory bowel disease.

5. The method according to claim 4 wherein said inflammatory bowel disease is Crohn's disease.

6. The method according to claim 4 wherein said inflammatory bowel disease is ulcerative colitis.

7. The method according to claim 1 wherein said disease is an arthritic condition.

8. The method according to claim 1 wherein said disease is sepsis.

9. The method according to claim 1 wherein said disease is lupus.

10. The method according to claim 1 wherein said disease is erythema nodosum leprosum.

11. The method according to claim 1 wherein said compound is administered orally or parenterally.

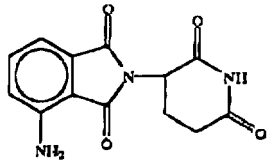
12. The method according to claim 1 wherein said compound is administered in combination with a therapeutic agent.

13. The method of claim 12 wherein said therapeutic agent is a steroid, antibiotic or neoplastic agent.

14. The method of claim 1 wherein said compound is administered orally.

15. The method of claim 1 wherein said compound is administered parenterally.

16. A method of treating an oncogenic or cancerous condition in a mammal which comprises administering thereto an effective amount of a compound of the formula:



or an acid addition salt thereof.

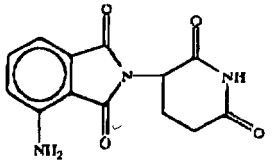
17. The method of claim 16 wherein said compound is administered orally.

18. The method of claim 16 wherein said compound is administered parenterally.

19. The method of claim 16 in which said compound is administered in combination with a therapeutic agent.

20. The method of claim 19 wherein said therapeutic agent is a steroid, neoplastic agent or antibiotic.

21. A pharmaceutical composition comprising, in combination with a pharmaceutically and physiologically suitable carrier, a compound of the formula:



or an acid addition salt thereof, in a quantity sufficient upon administration in a single or multiple dose regimen to a mammal to produce at least one of the effects of improving an oncogenic or cancerous condition, reducing inflammation, or improving autoimmune disease.

22. The pharmaceutical composition according to claim 21 in which said dosage form is selected from the group consisting of powder, tablets, capsules and injectable compositions.

23. The pharmaceutical composition of claim 21 which is administered in combination with a therapeutic agent.

24. The pharmaceutical composition of claim 23 wherein said therapeutic agent is a steroid.

25. The pharmaceutical composition of claim 23 wherein said therapeutic agent is an antibiotic.

26. The pharmaceutical composition of claim 23 wherein said therapeutic agent is an antineoplastic agent.

* * * * *

#9 attach

Sci. Pharm. 49, 67-99 (1981)

THE ARENE OXIDE HYPOTHESIS OF THALIDOMIDE ACTION. CONSIDERATIONS ON THE MOLECULAR MECHANISM OF ACTION OF THE "CLASSICAL" TERATOGEN*

H. Koch
Received June 14, 1980

It will soon be the twentieth anniversary of the day that marks an historic date, the event that has impressed itself on the consciousness of man as the "greatest pharmaceutical catastrophe of all time." On November 18, 1961, the suspicion was openly expressed for the first time** that thalidomide (or Contergan, as the preparation was called at that time) was the cause of the unusual frequency of severe and very severe deformities in newborn children that had appeared like an epidemic since about 1959 in various European and overseas countries (162, 189, 264).

Two decades have elapsed since then, and it gives food for thought that in this long period of time, in spite of intensive efforts, we have not been in a position to explain satisfactorily the molecular mechanism of action of this relatively simple compound. For that reason, it seems to be time for us to consider this problem again.

Over the twenty years, innumerable experimental data have been compiled, and the time may now have come when we can and should set about a critical examination and assessment of the findings. In the meantime so much circumstantial evidence has been collected that an explanation of the final cause of this unforeseen event which is unrivalled in the history of pharmaceuticals seems possible. The pieces of the mosaic are little by little being reconciled into a picture and the hope is justified that the pieces that are still missing will also soon be found and the last "blank spots" will be filled up!

The author who himself has been occupied with the thalidomide problem and the questions associated with it for many years (66, 130-142, 144, 145, 221-226, 259), is proposing a new working hypothesis and combines this with the desire that it will be a stimulus to further, targeted research and thus will be able to contribute to a definitive solution of the problem.

* Dedicated to Prof. E. Soos on his 70th birthday.

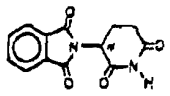
** As a discussion contribution by Prof. Widukind Lenz at a meeting of the Rhine-Westphalia Pediatrians Association in Dusseldorf, FRG.

Prior history

Thalidomide (Fig.1) came on to the market for the first time in the fall of 1957 and after a short time enjoyed extraordinary popularity as a tranquilizer and sedative, probably because it does not produce unpleasant after-effects ("hangover") like other hypnotics and because it possesses almost no acute toxicity (no danger of suicide).

The active substance thalidomide (INN) came on the market in many countries under numerous tradenames of which Contergan® was the best-known. The drug was given perorally (tablets, syrup) and rectally in a dosage of 12.5 to 100 mg. In May, 1960, 8.5 million, and in January, 1961, over 20 million daily doses were sold. About 700,000 citizens of the FRG took it regularly (279).

Thalidomide
"K-17," α-phthalimidoglutarimide



$C_{13}H_{10}N_2O_4$ MW = 258.23

CAS [50-35-1]

N-Phthalyl-glutamic acid imide
N-(2,6-Dioxo-3-piperidyl)-phthalimide
N-(2,6-Dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione

Fig. 1

However in the course of the years 1958-1961, at first singly and then ever more frequently, reports on side effects were received in which nerve damage ("thalidomide polyneuritis") was attributed to Contergan (84, 150). Then in November, 1961, when the suspicion was expressed that Contergan caused malformations in the unborn child ("Wiedemann syndrome," "thalidomide embryopathy," "dysmelia," etc.), the manufacturer immediately took all thalidomide-containing preparations off the market (162, 279).

Shortly afterwards, several hundred mothers came forward who had borne children without arms and/or legs, with hands directly attached to the shoulders and feet directly on the trunk ("seal limbs," "phocomelia") as well as with other more or less serious defects (eyes, ears, heart, etc.) (Fig. 2) and who stated that they had taken Contergan during their pregnancy. All together, worldwide, several tens of thousands of malformed children had been born and at least the same number had died non-viable (279).

That a causal relationship exists between the taking of thalidomide and the appearance of these so-called congenital malformations is not a question and is no longer seriously challenged by anyone. The obvious evidence for this is the correlation between the sales figures for Contergan, for example for the city of Hamburg (Fig. 3), and the increased occurrence of malformed newborns in the period from 1960 to 1962 at the same place. The path of the curves, except for a shift in time by nine months, is practically parallel and almost congruent. Similar curves also result for other places and for the whole FRG (160).

Fig. 2. Phocomelia

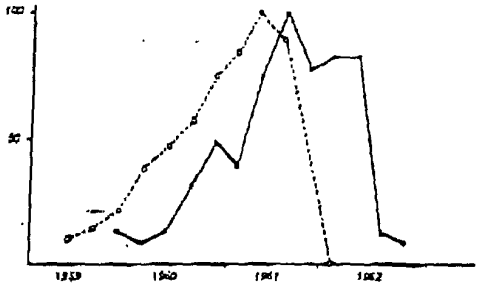
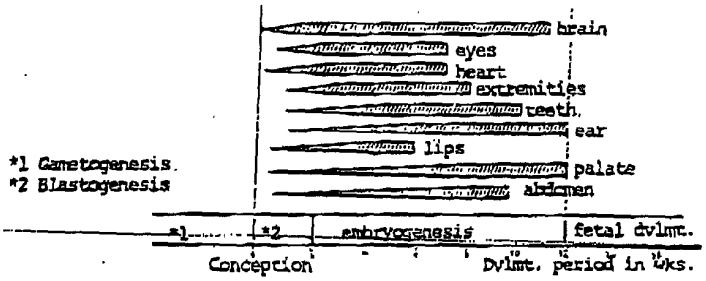


Fig. 3. Contergan sales and malformations

It is highly specific: anomalies were only triggered by thalidomide when it was taken at the so-called "sensitive phase." This phase of the embryonic development is in itself very brief: it is only 18 days, from the 34th to the 52nd day after the last menstruation, which corresponds to the period from the 24th to 42nd day after conception. Before and after that thalidomide had no teratogenic action. Within this sensitive phase there is a strict "timing" for the occurrence of certain malformations on the extremities and organs (155, 156, 158, 159, 242) (Fig. 4).

Fig. 4. Sensitive phases of embryonic development for thalidomide.



The following fact, which has already occasioned too much controversy, also appears noteworthy: Not in all cases in which thalidomide had demonstrably been taken by the mother-to-be were malformed children born (118, 161, 219, 242). Lenz, addressing this phenomenon characterized the "penetrating power as somewhere between 50 and 100 percent" (161).

Thus it seems that a specific (genetic?) disposition is necessary in the mother and/or child, possibly also a "second noxious agent" (128) as an additional damaging factor for the thalidomide teratogenesis.

Is there a specific "thalidomide receptor?

But what is this factor? That it is identical with a specific biological reaction partner of the thalidomide molecule in the organism, the hypothetical but to the highest degree specific "thalidomide receptor," is self-evident.

The search for the thalidomide receptor over all these years has only been accompanied by moderate success. Numerous speculations have been made and a multitude of original theories of action have been proposed, but none of them brought the longed-for explanation. They mostly did not withstand followup testing by experiment or remained unproved.

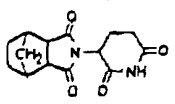
Here, therefore, is only a key-word-like listing of the most important of these thalidomide theories. We must forego a more detailed discussion of the proposals and experimental findings here. The interested reader is referred to the original literature.

- Glutamic acid antagonism (21, 50, 52-54, 72, 104, 149, 151, 179, 198, 204, 213, 228, 229, 235, 252)
- Folic acid antagonism (46, 54, 76, 151, 204, 213, 228, 243, 278)
- Vitamin anatagonism (21, 45, 50, 51, 62, 72-74, 76, 110, 125, 126, 191, 192, 206, 228, 234, 235, 267)
- Acylation hypothesis (24, 29-31, 49, 58, 63, 239, 245)
- Immunosuppression (18, 22, 28, 33, 37, 38, 44, 47, 67, 71, 82, 87, 98-100, 109, 157, 200, 201, 205, 227, 270, 277)
- DNA intercalation (23, 63, 77, 103, 144, 255)
- Proline hydroxylase inhibition (19, 152, 209, 211, 212)
- Disturbance of ossification (163, 164, 230-233)
- Embryonic peripheral neuropathy (178, 180-184)
- Endocrine dysregulation (14, 24, 80, 88, 166)
- Mesoderm damage (240, 281, 282)
- Inhibition of cellular respiration, anemia, hypoxia (12, 35, 43, 45, 52, 73, 83, 86, 125, 126, 220, 289).

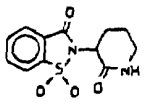
There were curious ideas among these, such as the grandiose speculation of Hellmann et al. (98-100, 270), which was refuted long ago (22, 33, 37, 157, 227, 277), but which always haunts some people, and those, based on serious considerations, such as a vitamin or aminoacid antagonism, but that have not been successfully proved or disproved.

The acylation hypothesis has been seriously discussed and worked on, but has since been clearly refuted (63, 107), and finally the DNA intercalation hypothesis, which we ourselves favored for a long time (136-142, 221, 222, 224, 225, 259). According to the most recent findings it likewise appears not to be tenable, but nevertheless as an (albeit false) working hypothesis it has contributed not insignificantly to the development of the non-teratogenic thalidomide analogs Taglutimide (1, 66, 130-142, 145, 221-226, 259) and Supidimide (9, 101, 102, 106, 250, 284) (Fig. 5).

Taglutimide and Supidimide



Taglutimide
(Biglumide, K-2004)
[CAS 14166-26-8]



Supidimide
(CG-3033, EM-87)
[CAS 49785-74-2]

Fig. 5

Structural requirements for teratogenicity

It is in any case established that certain structural features of the teratogenic substance - there is a series of thalidomide analogs, that exceed the original thalidomide in their teratogenicity, sometimes by a multiple factor (Fig. 6) - are essential for the embryotoxic and teratogenic activity. Certainly it is also found that these structural characteristics are absent from the analogs of thalidomide that have proved to be non-teratogenic (Fig. 7).

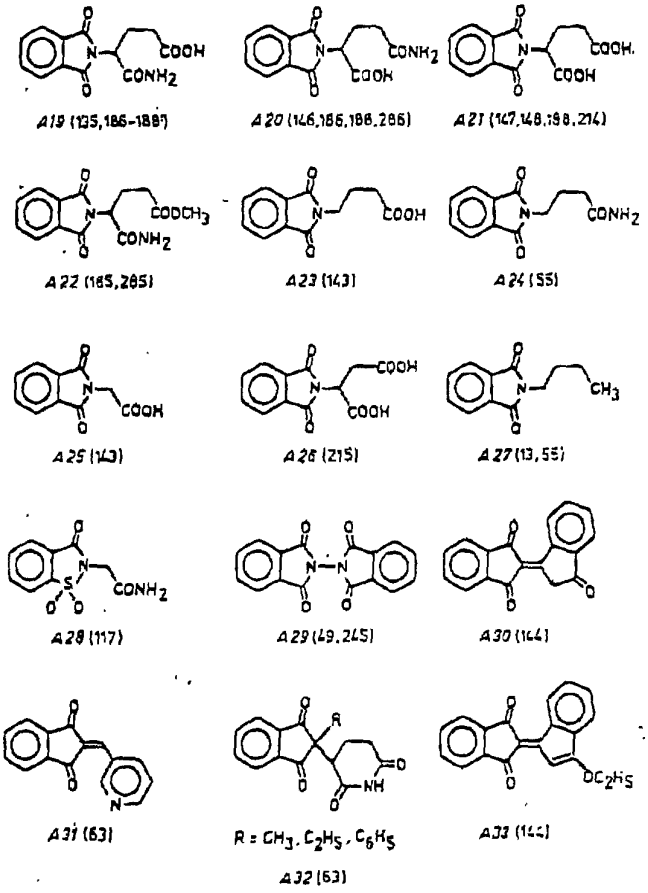


Fig. 6. Teratogenic thalomide analogs.

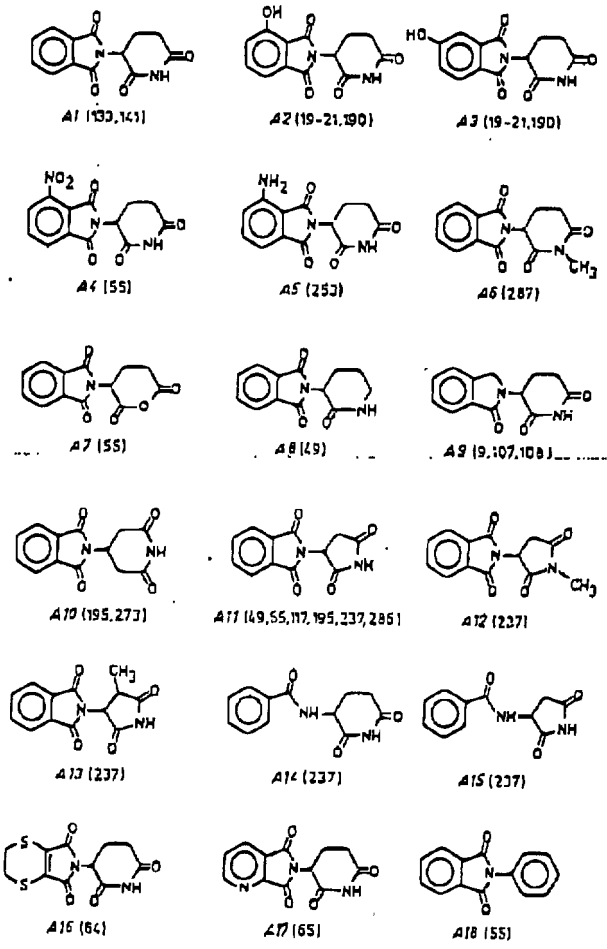


Fig. 6 (continued). Teratogenic thalidomide analogs.

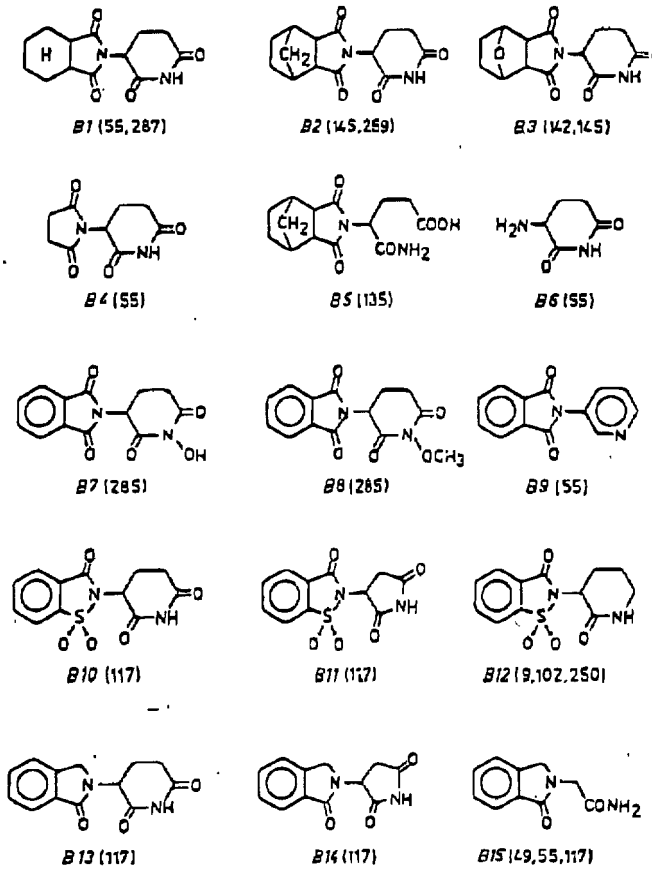


Fig. 7. Non-teratogenic thalidomide analogs.

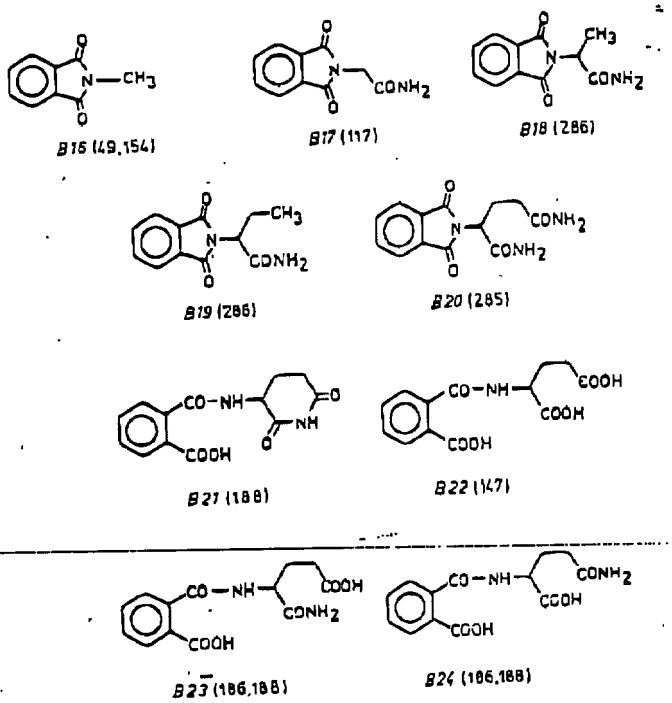


Fig. 7 (continued). Non-teratogenic thalidomide analogs.

In any case, we have here a starting point for our search for the causative principle. The fact that the search is occasionally diverted onto a false trail - cf. the DNA intercalation hypothesis! - must also be accepted in science. Once we have identified the principle of action, however, it is then usually also easy to identify the location of action and finally the receptor.

The study of the structure-effect relationships is a frequently practiced and (more rarely) successful method for the explanation of mechanisms of action in drugs. Therefore, many researchers, including us, at first pursued this course. A long time ago we were able to derive from the then available observations the "teratogenically effective minimal structure" (Fig. 8) for thalidomide-like compounds (133). This has not so far needed to be corrected in its essential content.

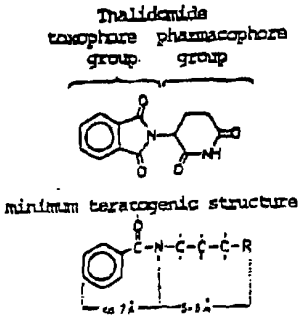


Fig. 8

For the realization of the teratogenic effect, the aromatic ring system (phthalimide, benzamide, etc.) is essential. The planarity of the latter, which was for a long time held to be essential (117, 133-140, 142, 144, 145, 221-226, 259), may however play no part (63).

The side-chain or the glutarimide ring evidently participate essentially in the bonding to the receptor. Compounds that do not fulfill these structural specifications (Fig. 8), or do not fulfill them sufficiently, are not teratogenically active. Detailed discussions of the structure-activity relationship in thalidomide and related compounds are found in (51, 117, 133, 244, 253). Here, it is sufficient for us to maintain for the present that the aromatic structure is the basic requirement for the teratogenicity.

As for the rest, the soporific and sedative therapeutic activity is definitely linked to the intact glutarimide ring (Fig. 8).

Biotransformation to an "active metabolite"

How does the specific biological reaction occur that finally leads to the teratogenic effect? We must start from the fact that it is not the original thalidomide molecule itself that engenders this reaction. Rather, the latter first of all experiences in the organism a completely specific change that first makes it capable of reacting with a biologically important macromolecule, for example a nucleic acid, a protein, a membrane constituent, etc. The by nature inactive thalidomide molecule must first be shifted to a "reactive state." This bioactivation is the key to the explanation of the teratogenicity.

It is not to be assumed that nature has reserved a completely private metabolic path to thalidomide. We therefore need only look around at what changes aromatic compounds in general are subject to in the higher organisms. In this way we encounter the ubiquitous metabolism of aromatic compounds by the oxygen-transferring microsomal enzyme system, the most important components of which are arylhydroxylase (AHH) and epoxidehydrase (EH) (40, 114, 216, 217).

To illustrate the determining processes, we consider the metabolism of bromobenzene, one of the best studied simple aromatics (Fig. 9), which will be representative of all those aromatic compounds that are subjected to similar biotransformations and that can therefore trigger comparable biological effects (25, 40, 114).

Metabolism of bromobenzene

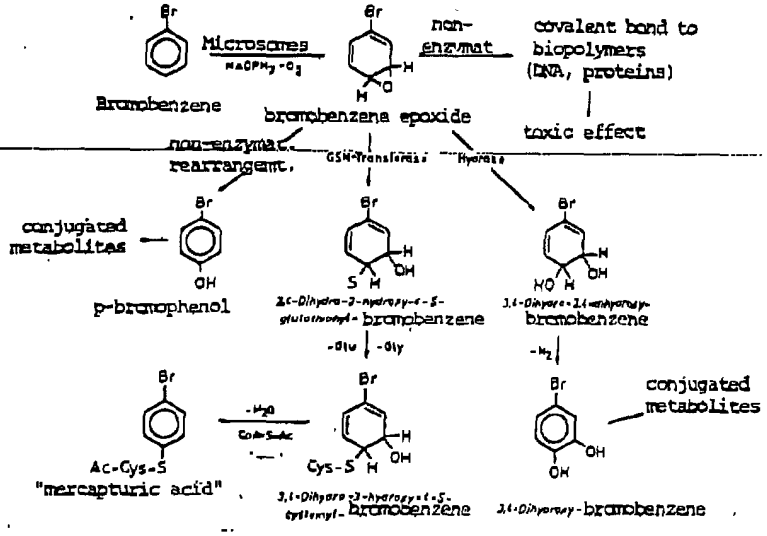


Fig. 9

It is quite conceivable that the aromatic thalidomide undergoes analogous changes in the organism. Numerous experimental findings point to this. It can be assumed that it initiates its toxic effects in this way, as is known from many other aromatic compounds. However these effects may be referred to - as hepatotoxicity, embryotoxicity, teratogenicity, mutagenicity, carcinogenicity, immunosuppression, cytostatic effect, etc. - they can all be traced back to the same primary cause, the interaction of a reactive metabolic intermediate with an important biological macromolecule.

In the case of bromobenzene, the fate of this compound in the organism has been studied in particular detail, and it is now known that its toxic effects (primarily hepatic necrosis) are elicited by an active metabolite. This metabolite arises on oxidation by the microsomal enzyme system of the liver and other organs; it is a simple epoxide.

It seems to be a faulty design of nature; The same active metabolite that is provided as the basis for detoxification at the same time is shown to be a powerful toxin for the cells. The process that should by rights protect the organism from damage under some circumstances becomes its doom. The path between detoxification and toxicity is a dangerous journey on a knife-edge!

Continuing with detoxification, it is possible in different ways, with, as stated, the reactive epoxide (arene oxide) representing the key point and the branching site.

The epoxide is the starting point for the mono- and di-hydroxylated metabolites which as hydrophilic compounds can be more easily eliminated than the lipophilic parent compound and which can, in addition be made to be still more intensively eliminated in the urine and bile by conjugation with glucuronic acid, sulfuric acid, aminoacids, etc.

The predominant route to detoxification in the case of bromobenzene is by coupling with glutathione (GSH) and conversion over several intermediate steps into an N-acetyl-cystaine conjugate, a so-called mercapturic acid (Fig. 9).

In this connection, it seems important to establish that the detoxification capacity of the organism is limited. In the mercapturic acid synthesis, for example, the actual GSH concentration is determining for the relative amounts of hydroxylated and conjugated metabolites (non-toxic metabolic products) and those of the free epoxide, which is bound covalently to macromolecules (and thus causes the toxic action). The conjugation due to the 9000-g fraction of the rat liver rises with the GSH content, while if the GSH is deficient the extent of covalent bonding increases enormously (Fig. 10) (116, 288).

There is a threshold ("GSH threshold") for the efficacy of this detoxification route. On the other hand, on flooding with the toxin the supply of conjugation agents is rapidly exhausted, so that the danger of toxicity becomes acute.

It would not be amiss to assume that the relations between the other detoxification routes are similar. The supply of "active sulfate" (PAPS), "active glucuronic acid" (UDPGA), "active methyl" (SAM) etc. is also not inexhaustible.

The remaining reactive epoxide now arylates certain macromolecules, presumably nucleic acids (DNA, RNA) and/or proteins (enzymes) by bonding to them irreversibly, thus forming a covalent bond to the acceptor.

It is obvious that such an irreversible arylation is equivalent to a denaturing of the biopolymer, which is not compatible with its normal function. A phenomenon of this kind must lead with directly conclusive consequences to a serious disorder.

Consider this further. Theoretically, a single thalidomide molecule on reaction with a nucleobase in an important section of the DNA already affects the replication of a cell in division so much that subsequently a malformation occurs.

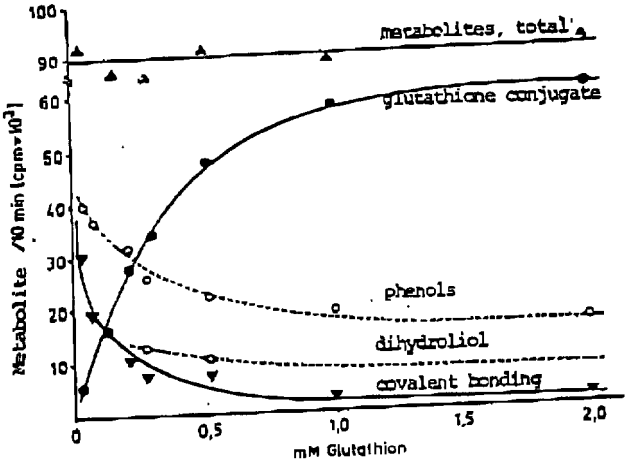


Fig. 10. In vitro metabolism of bromobenzene by rat liver homogenate in the presence of varying amounts of GSH.

In any case, assuredly, just a few of these "lucky hits", that is, a very few molecules of the active teratogen, are enough to trigger massive defects in the sensitive phase of embryonic development. However, when the natural repair mechanisms and detoxification routes are overtaxed by an excessive supply of metabolites, their protective function is sure to break down.

Here are a few more facts that support this hypothesis for the mechanism of the thalidomide effect. Bromobenzene will again be used as the model, but the general applicability of the statements for aromatic compounds may not thereby be disregarded.

Bromobenzene or its active metabolite is bound tightly to certain hepatic structures (25). Pre-treatment with enzyme inductors, such as for example phenobarbital, intensifies this effect and lowers the plasma level of the halohydrocarbon. The simultaneous administration of inhibitors of cytochrome P 450 such as Proadifen (SKF 525 A) or piperonyl butoxide (Fig. 11) raises the plasma level of the bromobenzene and lowers the fraction of the covalently bound aromatics in the liver (196). This is the evidence for the participation of the arene oxide in the genesis of necroses of the liver.

Inductors and inhibitors
of the drug-metabolising enzymes

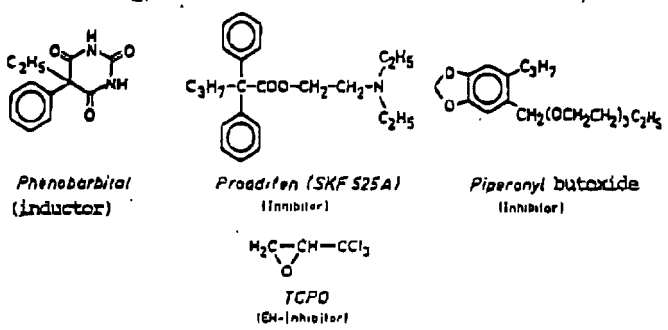


Fig. 11. Inductors and inhibitors of the enzyme system metabolizing foreign substances

Transitory liver damage with carbon tetrachloride

In the case of thalidomide, there is solid evidence that a similar mechanism is involved in the realization of its activity. This is the transitory liver damage with carbon tetrachloride already known for a long time but not correctly assessed as to its significance.

As early as 1964, Heine et al. (95) suspected that "in the genesis of malformations by thalidomide an as yet unknown factor that is perhaps related to the process of detoxification of thalidomide in the organism" is active. Their experiments on rabbits with CCl_4-damaged livers indicate the special part played by the liver in the genesis of the well-known abnormalities.

Heine et al. (94-97) were able to produce, after prior liver damage with CCl_4 in rabbits, a high percentage (> 80%) of embryonic malformations with thalidomide, whereas this had previously not been possible or not to as great an extent. Later, we too were able by using CCl_4 in rabbits to elicit almost 100% malformations with thalidomide (142, 259). Supplemental administration of ATP made it possible to partially antagonize the damaging effect of the thalidomide/CCl_4 combination (97).

This experiment shows clearly that liver damage with CCl_4 represents an additional noxious agent (128) which enormously raises the penetrating power of the thalidomide; during this, it is to be maintained that CCl_4 alone does not have a teratogenic effect (94-96), 249, 266) and that ATP may be able to negate the result of liver damage within certain limits. The ATP is evidently necessary for the subsequent conjugation reactions during the detoxification. The ATP deficiency occurring with the CCl_4 damage to the liver and the associated metabolic disturbances on the other hand accentuates the full teratogenic effect of the thalidomide (97).

The liver damage due to CCl_4 as is known meanwhile, does not disturb the degradation of the thalidomide occurring mainly by the hydrolytic route. Heine and Stüwe (97) found no significant difference in the blood levels (of unchanged thalidomide!) between rabbits with diseased livers and those with healthy ones. Their conclusion from the results of this experiment that "a disturbance in the degradation of the thalidomide does not occur as a result of liver damage" might of course have been hasty.

It is known from the most recent studies that the metabolites of CCl_4 are also bonded covalently to the DNA, the proteins, and the lipids in the cell nucleus (42). Why should this not also be the case with thalidomide?

The fate of thalidomide in the mammalian organism

At this point we must flash back briefly to the fate of thalidomide in the animal and human organism. The pharmacokinetics and metabolism of the substance have been very thoroughly studied by several groups of authors (10, 11, 53, 54, 60, 61, 90, 124, 246, 248, 263), and the results explain a few phenomena that are related to the particular teratogenicity of the substance.

After peroral administration, thalidomide is rapidly and practically completely absorbed and distributed fairly uniformly in the body. Experiments on pregnant animals (mouse, rat) showed that ^{14}C-labelled thalidomide easily penetrates the placental barrier. The radioactivity found in the fetus is comparable to that in the mother animal.

The pharmacokinetics also show that thalidomide and its metabolites are mainly excreted in the urine. 24 hours after p.o. application of 10 mg/kg ^{14}C-thalidomide in rats, two-thirds of the dose administered was eliminated in the urine, while only about 10% was contained in the feces. After 96 hours, 93.3% of the activity was found again in the urine and feces combined.

In the organism, thalidomide is subject to a simple hydrolytic degradation, whereby all twelve conceivable cleavage products appear. We ourselves have synthesized all the compounds shown in Fig. 12 and have developed a rapidly realized, sensitive TLC method for their detection (223).

The simple hydrolysis products, unlike the lipophilic parent substance, are ionizable, hydrophilic compounds (mono-, di- and tricarboxylic acids). At physiological pHs, they exist as hydrated ions which still have only a greatly diminished penetration capacity for the lipid-rich, biological membranes. The hydrolytic degradation goes fairly quickly: at pH 7.0, 7.4 and 8.0, half-lives of 11.5 and 1.25 hours were reported for the spontaneous hydrolysis of thalidomide (247).

The "mousetrap" hypothesis

This fact led early on to the conjecture that it was not the intact thalidomide molecule but one or more of its metabolites that were responsible for the teratogenic activity (119). The thalidomide molecule, according to this hypothesis, would only function as a transport-form for the teratogenic factor, which in this way would be "smuggled" into the embryo. Since the thalidomide that penetrates into the embryo is subject to the same hydrolytic degradation as in the maternal organism, the metabolites formed on the spot would be caught as though in a "mouse trap" and could no longer leave the embryo (56, 119, 121, 123, 244).

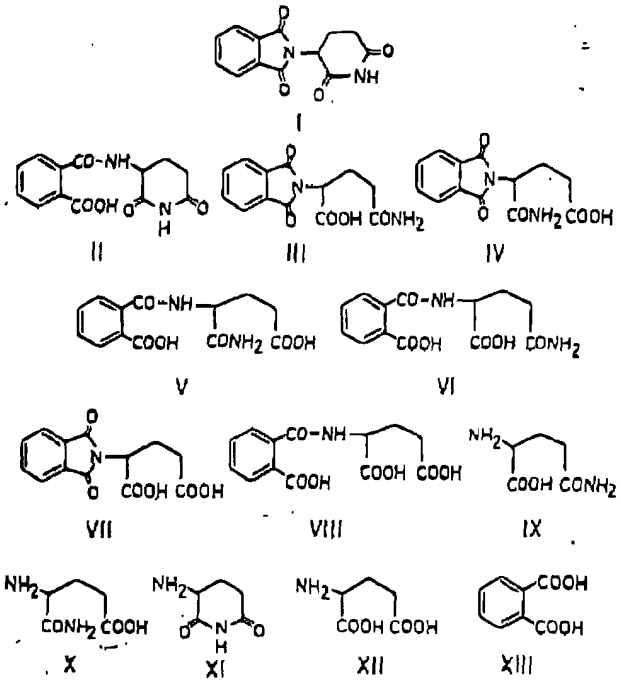


Fig. 12. Thalidomide and its metabolites

There is no doubt that this enrichment of hydrophilic and also teratogenic metabolites (135, 146-148, 186-188, 214, 286) in the embryo also raises the concentration of reactive epoxide at the site of action itself. Presumably this increase in concentration of the teratogenic agent is so great that in this way the natural detoxification and repair mechanisms are overtaxed and thus become ineffective. Certainly, in the realization of the thalidomide action a pharmacokinetic factor plays a not unimportant part.

Hydroxylated metabolites of thalidomide

There is still another important indication that argues for the postulated mechanism of thalidomide teratogenicity. Besides the simple hydrolytic cleavage products so far mentioned here (Fig. 12), in the mammalian organism nuclear-hydroxylated thalidomide metabolites also arise, only minor in quantity, but analytically unambiguously detectable. Derivatives of 3- and 4-hydroxyphthalic acid were identified in the urine of the experimental animals by their characteristic fluorescence (248, 254, 280).

The presumption that these hydroxylated metabolites of thalidomide represent the essential teratogenic principle had in any case been suspected relatively early (190) without however clear proposals having been developed on the molecular mechanism. Only a vague glutamic acid antagonism was considered (19, 21) and the possibility of an interaction with vitamins (thiamine, pyridoxal) was suggested (110).

The appearance of hydroxyphthalic acid supports our hypothesis on the reactive metabolites. In the hydroxyl derivatives we do not perceive the actual teratogenically active substance, but rather the non-toxic secondary products of the latter, since the real active compound is the unstable intermediate epoxide.

This of course does not mean that the hydroxylated metabolites have already lost their teratogenic potential completely. The 3-and 4-hydroxyphthalic acid derivatives can definitely still be subject to a further attack of the microsomal enzyme system, as also happens in the bioactivation of the polycyclic aromatic hydrocarbons (PAH) of the benzo[a]pyrene type (Fig. 13) (40, 114).

In fact it could be shown that the easily water-soluble 3- and 4-hydroxyl derivatives of thalidomide, for example, inhibit the growth of cell cultures from mouse embryos in vitro. The synthesis of protein and RNA in the cells is lowered significantly under their influence (194). Also in incubated chicken eggs they elicit a malformation rate comparable with that of thalidomide (19, 21), with the 4-hydroxy compound being clearly superior to the 3-hydroxy derivative in its activity (21).

The suspicion suggests itself that the original thalidomide molecule to some extent is the pre-teratogen which eventually is transformed by way of one or more intermediate metabolic steps, the proximal teratogens, to the ultimate teratogen.

Expressed simply, the process could be played out as follows: The reactive epoxide (possibly also a further diol epoxide) forms as an intermediate a carbonium ion which attacks a suitable acceptor in a nucleophilic reaction. In the normal case, this acceptor will be a water molecule. The resulting diol can then be spontaneously stabilized to the hydroxy derivative referred to (Fig. 14). A reaction with GSH is also conceivable (cf. Fig. 9).

In the specific case, the reactive intermediate step in all probability attacks a nucleic acid. There are grounds for the assumption that the exo-situated amino group of guanine reacts preferentially with the epoxide with formation of a covalent N-aryl derivative (Fig. 14) (111).

Bioactivation of benzo[a]pyrene

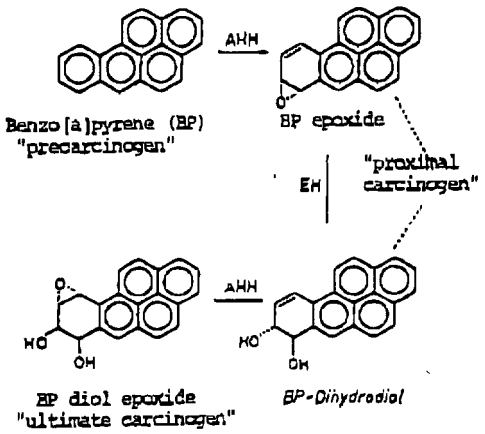


Fig. 13 AHH = aryl hydrocarbon hydroxylase (EC 1.14.14.2)
EH = epoxide hydratase (EC 4.2.1.63)

Prerequisite for this kind of interaction is the formation of a reactive epoxide (arene oxide) or a correspondingly stabilized carbonium ion. Understandably, only sufficiently "long-lived" carbonium ions can react with the DNA nucleophiles present in low concentration.

We thus postulate that the active metabolite that is responsible for the teratogenicity of thalidomide and analogous compounds is an arene oxide.

Bioactivation and interaction with DNA

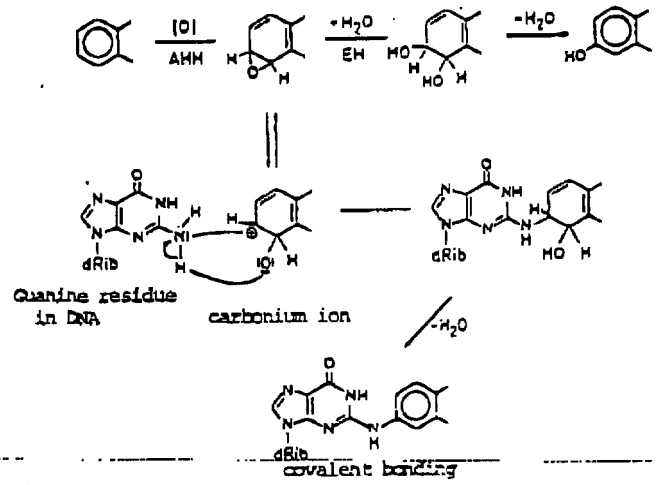
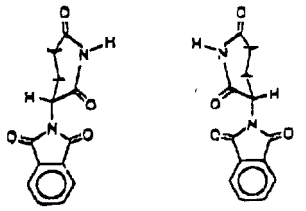


Fig. 14

Absolute Configuration



R-(+)-Thalidomide
$[\alpha]_D^{25} = +67°$ (DMF)

L-(-)-Thalidomide
$[\alpha]_D^{25} = -67.6°$ (DMF)

Fig. 15

This striking stereospecificity is not otherwise to be observed in the other effects of thalidomide. In their acute toxicity in the mouse, the two optically active forms do not differ from each other but each of them differs clearly from the racemate (59). On the other hand, the hypnosedative action is not significantly different in all three forms (59).

This finding encourages us in our opinion (142, 259) that the (desired) pharmacological activity of thalidomide (sedative and antiinflammatory action) and its unpleasant side effects (embryotoxicity, teratogenicity, neurotoxicity) are based on interactions of the active substance with different receptors.

Going back to the initial question: the quite specific structure and conformation of the S(-)-thalidomide molecule, as shown in Fig. 15, represents a mirror image of its bonding site, that is, of the hypothetical thalidomide-receptor, which in our opinion is identical with the active center of the enzyme on which the activation of the molecule takes place. All the innumerable aromatic compounds that do not display this additional structural feature and cannot fit this receptor are therefore not active in this way.

The high degree of stereospecificity of the teratogenic effect has its counterpart in the carcinogenicity of the PAH. Of the many oxidized metabolites of benzo[a]pyrene, for example, only one, i.e. the (-)-enantiomer of BP-7,8-dihydrodiol, and the diol epoxide formed from it (Fig. 13), possesses outstanding mutagenic and carcinogenic activity. And precisely this diol epoxide is preferentially formed in vivo (115, 174, 283). The persuasive power of this analogous model can hardly be denied.

Stereospecificity of the biotransformation

The properties of thalidomide summed up under the overall concept of "biological activity" are thus determined by its stereochemistry. So far, there are no reports on a different biotransformation of R(+)- and S(-)-thalidomide. We must therefore have recourse to inferences by analogy if we wish to support our assumption that the (oxidative) metabolism of the enantiomers also goes differently.

If the evidence is obtained that the S(-)-form is converted to a reactive arene oxide, but the R(+)-isomer is not, then this would supply the proof that the long-sought-after thalidomide receptor is identical with the arylhydroxylase. As we have said, we can only suspect this at the present time; the experimental proof of a stereoselective metabolism based on this working hypothesis should not, however, be long in coming.

There are numerous reports in the literature on stereoselectivity in the metabolism of drugs (112). We cite only two drugs, which are related to thalidomide by their imide structure, to illustrate this fact.

The phenytoin molecule has a prochiral center, that is, substitution on one of the two phenyl groups produces asymmetry in its middle C atom. In the metabolism, one phenyl group is selectively hydroxylated. The final effect is that a mixture of the optically active m- and p-hydroxy derivatives results (Fig. 16) (4, 26, 177).

The route by which these metabolites arise is clear from what has been said. The connecting link was found with the successful isolation of the optically active 5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-5-phenylhydantoin (Fig. 16) with a rotation of -163° (32). On heating with acid, the dihydroxy derivative converts to a mixture of the two phenols (32).

The provisional final link in the chain of evidence was contributed by an elegant work in the course of which it was possible to produce a correlation between the embyropathic effect of the phenyltoin and the assumed covalent bonding of the intermediate arene oxide to the macromolecules in the fetal tissue and in the placenta (15, 175).

Phenytoin, as stated, elicits dose-dependent teratogenic effects in the mouse. By simultaneous administration of trichloropropene oxide (TCPO, Fig. 11), an epoxidehydratase inhibitor, the rate of malformations is significantly raised. The (radioactively labelled) phenytoin is fixed in the fetus and in the placenta, and the amount of covalently bound teratogen in the tissues increases to the same extant as the number of malformations (175). Other inductors and inhibitors (phenobarbital, SKF 525 A) antagonize or potentiate the metabolism, and thus the teratogenic activity of phenytoin (39, 92, 262).

Is DNA the target of the ultimate teratogen?

In conclusion, we want to return to the question of whether the nucleic acid really represents the point of attack of the teratogen thalidomide. Fundamentally, there is no question: the indications that argue in favor of this are simply "overwhelming."

An interaction with nucleic acids was suspected fairly early and repeatedly (41, 77, 103, 272, 273) and a covalent bonding to certain cell structures was also considered (5, 6, 23, 57, 246).

Dannenberg and Sonnenbichler (41) found a distinct elevation of the "melting point" of the DNA due to thalidomide. They considered the relationship of the thalidomide teratogenicity with the PAH carcinogenicity in some way, but could not establish the relationship (41).

Thalidomide is, however, not merely teratogenic in the womb, it also elicits embryotoxic effects in the offspring when the male parent is treated with the teratogen (86, 105, 170, 172, 173). This kind of effect can only occur due to damage to the genetic apparatus of the germ-cells.

In many kinds of test systems thalidomide exerts a cytostatic effect (18, 38, 79, 165, 194, 198, 202, 203, 235, 260, 265, 271, 274-276). This is a manifestation of the inhibiting action on the function of the DNA and is causally identical with the teratogenic action. Thalidomide also inhibits RNA synthesis in different biological systems (48, 194, 210).

Finally, thalidomide is also carcinogenically active. It intensifies the oncogenesis due to methylcholanthrene (197), and in one case even alone triggers local tumors in the mouse (236). Thus the causative relation between teratogenicity and carcinogenicity is self-evident.

Why are Taglutimide and Supidimide not teratogenic?

As a supplement and appendix to the above considerations, one more comment/statement "in our own cause."

Of the thalidomide analogs synthesized in great numbers and in some cases tested, so far only two (non-teratogenic) candidates raise a claim to finding admission into therapy as successor preparations to the erstwhile drug thalidomide, Taglutimide and Supidimide (Fig. 5).

Taglutimide was developed by us (130-132), Supidimide by the then producer of thalidomide (284). Both research groups, indpendently of each other, came to the conclusion that it would be worth while and also justifiable to prepare an active substance that would retain the advantageous therapeutic properties of the original thalidomide without displaying its undesirable side effects. These considerations are in the meantime shared by other observers (75).

Both preparations, Taglutimide and Supimide, had in the meantime been very thoroughly and responsibly tested in decade-long work. They are in all probability suitable as effective and non-damaging drugs for use in human therapy.

Taglutimide does not contain an aromatic ring; bioactivation to a toxic metabolite according to the "arene oxide hypothesis" is not possible. However, it still contains the intact glutarimide structure that was known as the "pharmacophore" or "therapogenic" grouping (see Fig. 8).

1 G. Argyropoulos, G. Lanner und E. Pasquali: Wien. Med. Wochenschr. 126, 75 (1976).
2 F. H. Allen und J. Trotter: Chem. Commun. 1970, 776; J. Chem. Soc., Ser. B 1971, 1073.
3 J. D. Amirkhanian: Experientia 26, 798 (1970).
4 A. J. Atkinson, J. MacGee, J. Strong, P. Garteiz und T. E. Gaffney: Biochem. Pharmacol. 19, 2483 (1970).
5 B. Bakay und W. L. Nyhan: J. Pharmacol. exp. Ther. 161, 348 (1968).
6 B. Bakay und W. L. Nyhan: J. Pharmacol. exp. Ther. 171, 109 (1970).
7 C. Baroche: Bull. Cancer 52, 241 (1965).
8 C. Baroche: Bull. Cancer 55, 413 (1968).
9 A. S. Bazzoli, J. Manson, W. J. Scott und J. G. Wilson: J. Embryol. exp. Morph. 41, 125 (1977).
10 R. Beckmann: Arzneim.-Forsch. 12, 1095 (1962); 13, 185 (1963).
11 R. Beckmann: Arch. exp. Path. Pharmakol. 245, 90 (1963).
12 A. V. Beran, J. Strauss, O. R. Sperling, A. C. Norton, V. P. Garwood und J. Yamagami: Pediat. Res. 5, 199 (1971).
13 G. Bignami, G. Bovet, F. Bovet-Nitti und V. Rosnati: Lancet 1982, II, 1033.
14 G. Bignami und C. R. Cartoni: Farmaco, Ed. Sci. 22, 90 (1967).
15 D. A. Blake, F. Martz, C. Failinger und A. M. Gery-Martz: Carcinog.-Compr. Surv. 1978, 401.
16 G. Blaschke, H. P. Kraft, K. Fickentscher und F. Köhler: Arzneim.-Forsch. 29, 1640 (1979).
17 G. Blaschke, H. P. Kraft und H. Markgraf: Chem. Ber. 113, 2318 (1980).
18 R. Böhm und K. Nilson: Lancet 1986, I, 92.
19 J. B. Boylen, H. H. Horne und W. J. Johnson: Lancet 1963, I, 552.
20 J. B. Boylen, H. H. Horne und W. J. Johnson: Proc. Can. Fed. Biol. Soc. 6, 12 (1963).
21 J. B. Boylen, H. H. Horne und W. J. Johnson: Can. J. Biochem. 42, 35 (1964).
22 P. J. Bore und R. J. Scothorne: Lancet 1955, I, 1240.
23 S. Borghi, C. Botre, P. Cicconetti und P. Mazzeo: Farmaco, Ed. Sci. 21, 723 (1966).
24 E. Brode: Arzneim.-Forsch. 18, 561, 801, 1051, 1222 (1968).
25 B. B. Brodie, W. D. Reid, A. K. Cho, G. Sipes, G. Krishna und J. R. Gillette: Proc. Natl. Acad. Sci. USA 68, 160 (1971).
26 T. C. Butler: J. Pharm. exp. Ther. 119, 1 (1957).
27 A. S. Cahn, C. K. Ingold und V. Prelog: Experientia 12, 81 (1956).
28 P. Cerruti Mainardi, C. Borrone und A. M. Tambussi: Minerva Pediat. 15, 1392 (1963).
29 S. Champy-Hatem: Arzneim.-Forsch. 15, 508 (1965); 17, 1155 (1967).
30 S. Champy-Hatem: Bull. Acad. Nat. Med. 146, 301 (1964); 149, 426 (1965).
31 S. Champy-Hatem: Compt. rend. Acad. Sci. 257, 3138 (1963); 258, 1204 (1964); 260, 4208 (1965); 261, 271 (1965); 262, 18 (1966).

75 R. C. A. Fredrickson, I. H. Slater, W. E. Dusenberry, C. R. Howes, G. T. Jones und R. A. Moore: J. Pharmacol. exp. Ther. 203, 240 (1977).
76 L. Friedman, G. M. Shue und E. L. Hove: J. Nutrit. 65, 309 (1965).
77 S. Furberg: Acta Chem. Scand. 19, 1266 (1965).
78 S. Furberg und C. S. Pedersen: Acta Chem. Scand. 19, 253 (1965).
79 C. Gasparini, M. Bergi und S. Romito: Arch. Sci. Med. 128, 65, 69 (1971).
80 M. Getato, S. K. Quadri und J. Meites: Proc. Soc. exp. Biol. Med. 140, 167 (1972).
81 E. Genazzani G. Andreoli, R. DiCarlo und G. Pagnini: Toxicol. appl. Pharmacol. 8, 522 (1966).
82 E. Genovesi: Ann. Ottalmol. clin. oculist. 93, 811 (1967).
83 L. L. Gershbein: Biochem. Pharmacol. 14, 893 (1965).
84 E. Gibbels: Die Thalidomid-Polyneuritis, Verlag. G. Thieme, Stuttgart 1967.
85 J. E. Gibson und B. A. Becker: Proc. Soc. exp. Biol. Med. 128, 905 (1968).
86 C. N. Graves, G. W. Salisbury und J. R. Lodge: J. Animal Sci. 26, 1062 (1967).
87 J. P. Gusdon und C. Cohen: Am. J. Obstet. Gynecol. 100, 952 (1968).
88 E. Hagen, W. Wittkowski und P. A. Stöhr: Z. Krebsforsch. 74, 97 (1971).
89 E. Hagström und S. Lönning: Experientia 33, 1227 (1977).
90 D. E. Hague, S. Fabro und R. L. Smith: J. Pharm. Pharmacol. 19, 603 (1967).
91 H. Hanauske-Abel und V. Günzler: Z. Naturforsch. 32c, 241 (1977).
92 R. D. Harbison und B. A. Becker: J. Pharmacol. exp. Ther. 175, 283 (1970).
93 R. D. Harbison und B. A. Becker: Toxicol. appl. Pharmacol. 22, 193 (1972).
94 W. Heinz: Z. Kinderheilkunde 98, 141 (1966).
95 W. Heinz, H. Kirchmair, M. Fiedler und W. Stüwe: Klin. Wochenschr. 42, 592 (1964).
96 W. Heinz, H. Kirchmair, M. Fiedler und W. Stüwe: Z. Kinderheilkunde 91, 213 (1964).
97 W. Heinz und W. Stüwe: Z. Kinderheilkunde 98, 18 (1966).
98 K. Hellmann: Lancet 1958, I. 1136.
99 K. Hellmann: Wien. Med. Wochenschr. 117, 415 (1967).
100 K. Hellmann, O. I. Duke und F. Tucker: Brit. med. J. 1965, II. 687.
101 F. Helm, R. Becker, L. Flohé, E. Frankus, I. Graudumis, A. Hendrickx und W. Scott: 7th Int. Sympos. Med. Chem. Torremolinos, Spain 1980. S. 2180.
102 A. G. Hendrickx und F. C. Helm: Teratology 22, 179 (1980).
103 L. B. Hendry, F. M. Whitham und C. L. Chapman: Perspectives in Biology and Medicine 21, 120 (1977).
104 E. Hirschberg, M. Osnos, S. Bryant und J. E. Ullmann: Science 143, 1340 (1964).
105 S. M. Husain und M. Pellerin: Can. Med. Assoc. J. 103, 163 (1970).
106 T. M. Itil, M. Bozak, N. Polvan, C. Saldatos, D. M. Shapiro, V. Krynicki und V. Morgan: Curr. Ther. Res. 27, 442 (1980).
107 A. J. Jackson und H. J. Schumacher: Can. J. Pharm. Sci. 15, 21 (1980).
108 A. J. Jackson und H. Schumacher: Teratology 19, 341 (1979).
109 W. P. Jaffe: Veterinary Record 78, 883 (1966).
110 R. Jasmin und W. J. Johnson: Can. J. Microbiol. 12, 333 (1966).
111 A. M. Jeffrey, I. B. Weinstein, K. W. Jennette, K. Grzeskowiak, K. Nakanishi, R. G. Harvey, H. Autrup und C. Harris: Nature 269, 348 (1977).
112 P. Jenner und B. Testa: Drug Metab. Rev. 2, 117 (1973).
113 M. K. Jensen: Acta Med. Scand. 177, 783 (1965).
114 O. M. Jerina und J. W. Daly: Science 185, 573 (1974).
115 O. M. Jerina, H. Yagi, D. R. Thakker, W. Levin, A. W. Wood und A. H. Conney: Symposium on reactive metabolites and their implications for toxicology. 7th Int. Pharm. Congress, Paris 1978.
116 D. J. Jollow, J. R. Mitchell, N. Zampaglione und J. R. Gillette: Pharmacology 11, 151 (1974).
117 N. A. Jönsson: Acta Pharm. Suec. 9, 425, 431, 521, 545 (1972).
118 T. Kaji, M. Kida und K. Takahashi: Teratology 8, 153 (1973).

164 V. G. Leone und L. Rinaldi: Excerpta medica Int. Congr. Ser. 173, 23 (1967).
165 K. Lindahl-Kiessling und J. A. Book: Lancet 1953, II, 405.
166 O. Locker, E. Superstine und F. G. Suiman: Arch. int. Pharmacodyn. 194, 39 (1971).
167 P. M. Loughnan, H. Gold und J. C. Vance: Lancet 1973, I, 70.
168 C. R. Lowe: Lancet 1962, II, 1332.
169 H. Löers: Lancet 1962, II, 1332.
170 M. M. Lukkens: Am. J. Pharm. Educ. 35, 776 (1971).
171 K. G. Lüning und T. Jernudd: Opusc. Med. 12, 406 (1967).
172 C. Lutwak-Mann: Brit. Med. J. 1958, I, 1080.
173 C. Lutwak-Mann, K. Schmid und H. Keberle: Nature 214, 1018 (1967).
174 C. Malavetile, T. Kuroki, P. Sims, P. L. Grover und H. Bartsch: Mutat. Res. 44, 313 (1977).
175 F. Martz, C. Fallinger und O. A. Bigke: J. Pharmacol. exp. Ther. 203, 231 (1977).
176 K. M. Massey: J. Oral Ther. Pharmacol. 2, 38 (1965).
177 E. W. Maynert: J. Pharm. exp. Ther. 130, 275 (1960).
178 W. G. McBride: Teratology 10, 283 (1974).
179 J. O. McColl, M. Globus und S. Robinson: Can. J. Physiol. Pharmacol. 43, 89 (1965).
180 J. McCredie: Lancet 1973, II, 1058.
181 J. McCredie: Medical Hypotheses 2, 53 (1976).
182 J. McCredie: Med. J. Austral. 1974, I, 159.
183 J. McCredie und W. G. McBride: Clin. Radiol. 24, 204 (1973).
184 J. McCredie und J. G. McLeod: Lancet 1974, I, 1111.
185 W. P. McNulty und H. M. Wuest: Proc. 3rd Int. Congr. Primat. Zürich 1970, Verlag S. Karger, Basel 1971, Vol. 2, S. 236.
186 W. Meise und F. Köhler: Pharmazie 27, 418 (1972).
187 W. Meise und F. Köhler: Z. Naturforsch. 26b, 857 (1971).
188 W. Meise, H. Ockenfels und F. Köhler: Experientia 29, 423 (1973).
189 G. W. Mellin und M. Katzenstein: New Engl. J. Med. 267, 1184, 1238 (1962).
190 M. Ménard, L. Erlenmeyer-Ch, M. LaBrooy und F. L. Chubb: Can. J. Chem. 41, 1722 (1963).
191 T. Midtvedt: Acta Path. Microbiol. Scand. 58, 355 (1963).
192 T. Midtvedt: Tidsskr. Norsk. Laegeler. 83, 719 (1963).
193 J. H. D. Millar und J. C. Nevin: Lancet 1973, I, 359.
194 Z. B. Miller: Lancet 1975, II, 1068.
195 D. Misiti, V. Rosnati, G. Bignami, F. Soyer-Nitti und O. Soyer: J. Med. Pharm. Chem. 6, 464 (1963).
196 J. R. Mitchell, W. D. Reid, B. Christie, J. Moskowitz, G. Krishna und B. B. Brodie: Res. Commun. Chem. Pathol. Pharmacol. 2, 377 (1971).
197 M. Miura, C. M. Southam und H. Wuest: Experientia 26, 305 (1970).
198 T. Mohri und H. Kitagawa: Chem. Pharm. Bull. 16, 2259 (1968).
199 R. R. Monson, L. Rosenberg, S. C. Hartz, S. Shapiro, O. P. Heinonen und D. Slone: New Engl. J. Med. 289, 1049 (1973).
200 G. L. Mouzas: Lancet 1968, I, 882.
201 G. L. Mouzas und A. K. Gershon: Transplantation 6, 478 (1968).
202 H. Mückter: Antimicrob. Agents Chemother. 5, 531 (1965).
203 H. Mückter und E. Mohr: Arzneim.-Forsch. 16, 129 (1966).
204 M. L. Murphy, in: CIBA Foundation Symposium on Congenital Malformations, London 1960, S. 78.
205 G. P. Murphy, N. O. Breda, H. W. Weber, J. H. Groeneveld und P. O. Williams: J. Med. 1, 66 (1970).
206 E. C. Naber und E. J. Largent: Poultry Sci. 44, 1583 (1965).
207 A. T. Natarajan und R. Nilsson: Naturwiss. 53, 275 (1966).
208 G. H. L. Nefkens, G. I. Tesser und R. J. Nivard: Rec. Trav. Chim. Pays-Bas 79, 688 (1960).

249 B. A. Schwetz, B. K. J. Leong und P. J. Gehring: Toxicol. appl. Pharmacol. 28, 442, 452 (1974).
250 W. J. Scott, J. G. Wilson und F. C. Helm: Teratology 22, 183 (1980).
251 Y. F. Shealy, C. E. Dpilger und J. A. Montgomery: Chem. & Ind. 1965, 1030; J. Pharm. Sci. 57, 757 (1968).
252 R. L. Smith: Proc. 2nd Int. Pharmacol Meeting, Prague 1965, Bd. 4, S. 481.
253 R. L. Smith, S. Fabro, H. Schumacher und R. T. Williams: Studies on the relationship between the chemical structure and embryotoxic activity of thalidomide and related compounds. In: J. M. Robson, F. Sullivan and R. L. Smith (eds.): Embryopathic activity of drugs. J. & A. Churchill Ltd., London 1965, S. 194.
254 R. L. Smith, R. A. O. Williams und R. T. Williams: Life Sci. 7, 333 (1962).
255 J. Sonnenbichler und L. Lüling: Arzneim.-Forsch. 28, 1567 (1978).
256 J. South: Lancet 1972, II, 1154.
257 R. E. Staples, In: D. M. Woodbury, J. K. Penry und R. P. Schmidt: Antiepileptic Drugs, Raven Press, New York 1972, S. 55.
258 P. A. Stokes, P. H. Vardy, W. G. McBride und M. A. Long: IRCS Med. Sci. 7, 451 (1979).
259 L. Stockinger und H. Koch: Arzneim.-Forsch. 19, 167 (1969).
260 K. Sugiura und H. M. Wuest: Gann 55, 57 (1964).
261 F. M. Sullivan und P. R. McElhatton: Toxicol. appl. Pharmacol. 40, 355 (1977).
262 E. A. Swinyard, J. A. Madsen und L. S. Goodman: J. Pharmacol. exp. Ther. 111, 54 (1954).
263 A. Tanaka, A. Hasegawa und G. Urakubo: Chem. Pharm. Bull. 13, 1253 (1965).
264 N. B. Taussig: J. Am. Med. Assoc. 190, 1106 (1962).
265 B. P. Thomas, M. M. Ansari, M. R. Varia und G. N. Memon: Indian J. exp. Biol. 10, 314 (1972).
266 D. J. Thompson, S. D. Warner und V. B. Robinson: Toxicol. appl. Pharmacol. 29, 348 (1974).
267 A. Tolvanen, T. Markkanen, R. Mäniylärvi und P. Tolvanen: Biochem. Pharmacol. 13, 1489 (1964).
268 H. Tuchmann-Duplessis und L. Mercier-Parot: Compt. rend. Acad. Sci. 256, 1841 (1963).
269 A. Tumulka und R. W. Kaplan: Naturwiss. 52, 20 (1965).
270 J. L. Turk, K. Hellmann und D. I. Duke: Lancet 1965, I. 1134.
271 V. Vasilescu, D. Cilievici und L. Leanu: T. Cerc. Fiziol. 13, 293 (1968).
272 V. Vasilescu, A. Ciurea, C. Olmosiache und M. Constantinescu: Nature 202, 1080 (1964).
273 V. Vasilescu, E. Trutia und V. Topa: Naturwiss. 51, 333 (1964).
274 L. Villa und S. Eriani: Lancet 1963, I. 725.
275 L. Villa, S. Eriani, D. Tagliaretti und R. Valentini: Ricerca Sci. 3, 323 (1963).
276 L. Villa, R. Valentini, D. Tagliaretti und S. Eriani: Haematologica Latina 6, 217 (1963).
277 A. Vladutiu: Lancet 1966, I. 981.
278 M. Weber: Lyon Médical 235, 877 (1976).
279 O. Wenzel und K. H. Wenzel: Der Contergan-Prozeß, Bericht und Protokollauszüge. Presseabtlg. Thalidomid, Bensheim-Auerbach 1968.
280 R. T. Williams: Lancet 1963, I. 723.
281 E. Wolff: Ann. pharm. franc. 26, 475 (1968).
282 E. Wolff: Umschau 67, 354 (1967).
283 A. W. Wood, R. L. Chang, W. Levin, H. Yagi, D. R. Thakker, D. M. Jerina und A. H. Conney: Biochem. Biophys. Res. Commun. 77, 1389 (1977).
284 R. J. Woodhouse: Drugs of the Future 5, 449 (1980).
285 H. M. Wuest, R. R. Fox und D. D. Crary: Experientia 24, 993 (1968).
286 H. M. Wuest, I. Fratta und E. B. Sigg: Life Sci. 5, 395 (1966).